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Vol. LVI *June, 1929* *No. 6*

BIOLOGICAL BULLETIN

THE EFFECT OF CAFFEINE AND THEINE UPON THE EXCITABILITY OF THE SPINAL CORD.

ATTILIO RIZZOLO,

UNITED STATES BUREAU OF FISHERIES, WOODS HOLE, MASS.

In previous publications,[1] we have reported data concerning the effect of strychnine, cocaine, morphine and nicotine upon the excitability of the spinal cord in the selachians *Trygon vulgaris, Raja pastinaca, Scylliorhinus canicula.* Local applications of each alkaloid upon a motor point located on the dorsal side of the spinal cord first increased and later diminished the excitability of the motor point; local applications of one or other alkaloid on a motor point located on the ventral side of the cord caused no change in the excitability of the motor point. We have continued our study with the local applications of the alkaloids caffeine and theine.

The smooth dogfish (*Galeus canis* Mitchell) has served as subject of experiment. The size of the animal varied from fifty to sixty centimeters. Each alkaloid has been applied locally on the dorsal and ventral sides of the spinal cord. The excitability of the spinal cord has been determined before applications of one or the other alkaloid and after each application. The alkaloids have been prepared at a concentration of 2 per cent. in sodium chloride solution (8/1000).

For each alkaloid two series of experiment have been made. Each series consisted of six animals. In the first series, the dorsal side of the spinal cord was denuded in the region immediately anterior to the base of the posterior dorsal fin. In the second series, the ventral side of the spinal cord was exposed immediately

[1] See "Action de la strychnine sur les faces dorsales et ventrales de la moelle lombaire," by A. Rizzolo, *C. R. Soc. Biol.*, XCVI., 1927, p. 1207. "Effet de la cocaine de la morphine, et de la nicotine sur l'excitabilité de la moelle epinière," by A. Rizzolo, *C. R. Soc. Biol.*, XCVII., 1027, p. 1073.

anterior to the anal fin. In both series, the spinal cord was exposed to the extent of six millimeters. Motor points determining the movement of the posterior dorsal fin were located in the region immediately anterior to the base of the posterior dorsal fin; motor points determining the movement of the anal fin were located in the region immediately anterior to the anal fin. The excitabilities of the motor points located in each region were carefully measured in order to locate the motor point having the greatest excitability. We have designated the motor point having the greatest excitability as the optimum motor point. Local applications of caffeine or of theine were made on the optimum motor point and the excitability of the motor point was redetermined after each application. Briefly stated, the procedure of experiment was as follows:

1. The dorsal or the ventral side of the spinal cord was exposed.

2. The optimum motor point determining the movement of the posterior dorsal fin or of the anal fin was located; the excitability of the motor point was measured several times.

3. Applications of the alkaloid to be tested were made on the optimum motor point.

4. Immediately after each application, the excitability of the optimum motor point was redetermined.

DOGFISH V.—CAFFEINE.

Ventral side of the spinal cord immediately anterior to the anal fin. Measurements of the chronaxie made from the optimum motor point determining the movement of the anal fin.

A. Measurements Before Applications of the Alkaloid.

Rheobase (Volts).	Chronaxie (♂).
3.2	0.2
2.8	0.2
2.8	0.2
2.6	0.2

B. Measurements After Applications of the Alkaloid.

Rheobase (Volts).	Chronaxie (♂).
3	0.1
3	0.05
2.6	0.05
2.8	0.3
3.2	0.4
2.8	0.6

All animals have been operated without the employment of anesthetics. The animal was fastened to a cork board tilted so that the animal's head and gills remained completely submerged under water. The board was tilted in a large dissecting pan provided with a continuous flow of sea water.

The alkaloids were applied according to the method of Baglioni and Amantea. A piece of filter paper one and one half to two (1½–2) millimeters square was soaked in the solution of the alkaloid to be studied and was placed on the optimum motor point. Each application was made for a period of three minutes.

DOGFISH IV.—THEINE.

Ventral side of the spinal cord immediately anterior to the anal fin. Measurements of the chronaxie made from the optimum motor point determining the movement of the anal fin.

A. Measurements Before Applications of the Alkaloid.

Rheobase (Volts).	Chronaxie (♂).
3.4	0.3
3.4	0.3
3.2	0.3
3.6	0.3

B. Measurements After Applications of the Alkaloid.

Rheobase (Volts).	Chronaxie (♂).
3.4	0.1
3.8	0.1
3.6	0.2
4	0.6
3.8	0.7
3.6	0.9

The measurements of excitability were made in function of time according to the method of the "Chronaxie" devised by L. Lapicque. Employment of Lapicque's electrical rheotome "le chronaxiemetre" permitted the measurements to be made directly in thousands (1/1000) of a second—sigma. As positive electrode, Lapicque's modification of D'Arsonval's non-polarizable was employed; as negative electrode a silver wire was used. The negative electrode was plunged into the muscular tissue surrounding the region of the cord exposed. The positive electrode was held locally and placed intermittently by hand on a motor point whose excitability was being measured.

In this experiment, animals in which the optimum motor points measured chronaxies below 0.2 sigma were not accepted as subject of experiment.

Results and Conclusions.—The data obtained in each series of experiment for each alkaloid was concordant.

1. Four local applications of caffeine or theine at a concentration of 2 per cent. on the optimum motor point located on the dorsal side of the spinal cord and determining the movement of the posterior dorsal fin affected no change in the original chronaxie of the motor point. The motor point retained its original chronaxie after each application.

2. The first application of one or the other alkaloid on the optimum motor point located on the ventral side of the spinal cord and determining the movement of the anal fin diminished the original chronaxie from 33–67 per cent.; the fourth application increased the original chronaxie from 50–100 per cent. The sixth application affected an increase of even 200 per cent.

A case reporting the measurements made from the ventral side of cord is given for each alkaloid.

Summer, 1928.

A STUDY OF EQUILIBRIUM IN THE SMOOTH DOG-FISH—GALEUS CANIS (MITCHILL).*

ATTILIO RIZZOLO.

Sewell (1882) maintained it would be a bold assumption to assume the semicircular canals, ampullæ and vestibule (utricle and saccule(indispensable to the equilibrium of the selachians. Steiner (1886–1888) stressed the dispensability of these organs; Lee (1884–1886 contended their indispensability. Gaglio (1901) after bilateral ablation of a large part of the labyrinth obtained little or no disturbance of equilibrium. J. Loeb (1891) obtained disturbance of equilibrium following removal of the otolith from one saccule. G. H. Parker (1909) observed no disturbance following removal of the otolith from one or both saccules; section of both acoustic nerves caused profound disturbance of equilibrium. S. S. Maxwell (1910–1923) has observed that the animal swims quite normally after section of the two eighth nerves or after destruction of the two labyrinths. In the teleosts, Tomascewicz (1877) observed no disturbance of equilibrium after destruction of the semicircular canals and their ampulæ; Kiesselback (1882) cutting the horizontal canals on both sides obtained similar negative results.

In this paper we report our observations concerning the equilibrium of the *Galeus Canis* when the selachian was subjected to (1 Bilateral sectioning of the olfactory tracts,

(2) Bilateral sectioning of the optic nerves,
(3) Bilateral destruction of the labyrinths,
(4) Bilateral sectioning of the olfactory tracts, bilateral sectioning of the optic nerves and bilateral destruction of the labyrinths.

The animals were operated without the employment of local or general anæsthesia. Special care was taken to operate while the

* This investigation was undertaken at the U. S. Marine Laboratory, Woods Hole.

animal's head and gills remained submerged in sea water. The animal was fastened to a cork board tilted at an angle of 45° in a large dissecting pan provided with a continuous flow of sea water.

The behavior of the animals was studied in an aquarium 2¼ meters in length, 1¼ meters in width, and ⅘ meter in depth.

The experiments were limited to animals measuring 50 cm. to 75 cm. in length.

Operations.

Sectioning of the Olfactory Tracts.—Part of the cranial encasement (prefrontal and frontal regions) covering the olfactory tract and olfactory lobe was removed. A cavity was exposed which permitted sectioning of the tract. After sectioning the tract, the cavity was filled with cotton. The cotton was held in place by means of drawing stitches at right angles to each other immediately above it. No skin flap was made.

Sectioning of the Optic Nerves.—Cutting through the roof of the mouth a rectangular flap consisting of cartilage and palatal epithelium was made immediately over the junction (chiasma) of the optic nerves. The optic nerves were sectioned 1 mm.–1½ mm. from their place of junction. The flap was sutured into place again.

Destruction of the Labyrinths.—The approach to the labyrinth was made through the roof of the otic capsule. The destruction involved removal of the membranous vestibule (utricle and saccule, the three membranous semicircular canals and the three membranous ampullæ. The cartilaginous labyrinth corresponding to these parts of the membranous labyrinth was lacerated. The resulting cavity was thoroughly cleansed with cotton. After the cleansing, the cavity was filled with a cotton pack and the skin flap made over the roof of the capsule at the beginning of the operation was replaced and sutured.

Experiments and Results.

Bilateral Sectioning of the Olfactory Tracts.—Sectioning both olfactory tracts in the same animal caused no disturbance of equilibrium. Swimming remained normal. The animal died five to

six days after sectioning of the tracts. Six animals were operated upon.

Bilateral Sectioning of the Optic Nerves.—In the same animal, both optic nerves were sectioned. In two cases, swimming remained normal; in five cases movement in the horizontal plane was disturbed. In the latter cases, the animal at times swam around the dorso-ventral axis in circles or in the path of a curved line. Changing the direction of the animal changed the direction of its swimming. Turned to the right, the animal swam to the right around the dorso-ventral axis; turned to the left, it swam to the left. In other planes, the animal swam normally. Twelve to fifteen hours after sectioning of the nerves, the tendency to swim around the dorso-ventral axis disappeared; movement was normal in all planes. The animal died three to four days after the operation.

Bilateral Destruction of the Labyrinths.—In the same animal both labyrinths were carefully and neatly destroyed. In all cases, disturbances of equilibrium resulted. All animals died within three days. The cases may be grouped as follows:

1. Cases (6) in which movement was defective in the vertical and oblique planes to the surface of the water. In other planes, movement was normal. Rotation around the longitudinal axis when swimming in a plane vertical or oblique to the surface of the water never disappeared; it continued until the animal died.

2. Cases (14) in which the animal manifested unsteadiness in keeping the dorsal side up and difficulty in righting itself if placed on its back. The animal swam normally in all planes. At times its ability to keep the dorsal side up becoming critical, the animal would make a turn to the right or to the left towards the bottom of the aquarium. By so doing, it controlled its equilibrium and swam away normally—dorsal side up. Placed ventral side up at the surface of the water, the animal swam on its back a considerable length of time before righting itself. The righting movements were difficult. After a lapse of twenty-four hours, the tendency to be occasionally unsteady in keeping the dorsal side up disappeared; the animal continued to right itself with difficulty when placed on its back.

3. Cases (19) in which the animal retained temporary control

of equilibrium. At times, the animal swam normally in all planes; at other times it lost complete control of its equilibrium. When equilibrium was lost, the animal swam on its back, rotated around its axes, spiraled through the water and nose dived. Rotation around the axes and spirals were made indiscriminately to the right and left. After a period of disturbed equilibrium, the animal regained normal equilibrium only to lose it again sooner or later. The periods of normal and disturbed equilibrium were equally divided. Four to twenty-four hours after destruction of the labyrinths, movement was normal in all planes but if placed ventral side up at the surface of the water the animal swam on its back until it righted. In this respect, equilibrium remained disturbed.

4. Cases (11) in which the animal did not retain temporary control of equilibrium. Following the destruction of the labyrinths, equilibrium was lost for a period of four to twenty-four hours. Movement was disturbed in all planes. As in the case of the preceding group, the animal rotated around its axes, spiraled through the water, swam on its back and nose dived. Twenty-four hours after removal of the labyrinths, movement was normal in all planes. The animal's equilibrium was comparable to the equilibrium of the normal animal but for one exception. Placed ventral side up at the surface of the water, the animal righted itself with difficulty; it swam on its back until it righted. The difficulty in righting itself when placed on its back was always present; it did not disappear.

Bilateral Sectioning of the Olfactory Tracts, Bilateral Sectioning of the Optic Nerves and Bilateral Destruction of the Labyrinths.—In each animal both olfactory tracts, both optic nerves and both labyrinths were destroyed. The olfactory tracts were sectioned first. Two hours later the optic nerves were sectioned. When sectioning of the optic nerves caused the animal to rotate in circles or in the path of a curved line around the dorso-ventral axis, the labyrinths were destroyed after movement in the horizontal plane had become normal. When sectioning of the optic nerves permitted movement in the horizontal plane to remain normal, the labyrinths were destroyed four hours after sectioning of the nerves. The disturbances of equilibrium following destruction of the labyrinths were the same as the disturbances described

when only the labyrinths were destroyed; the improvements in the animal's movements following the disturbances of equilibrium were also the same. In brief, the same types of case were obtained when the destruction of the labyrinths was preceded by sectioning of the olfactory tracts and optic nerves as when only the labyrinths were destroyed. Nineteen animals were operated upon. In four cases, the animal rotated around the longitudinal axis when swimming to the surface of the water; in three cases, the animal manifested unsteadiness in keeping the dorsal side up and difficulty in righting itself when placed on its back; in seven cases, temporary control of equilibrium was retained; in four cases, equilibrium was lost for a period of four to twenty-four hours.

Conclusions.

1. *Bilateral Destruction of the Labyrinths Causes Disturbances of Equilibrium in All Animals.*—In some animals the disturbance is more marked than in others. When the disturbance is not very marked, the animal either rotates around its longitudinal axis when swimming to the surface of the water or swims on its back and rights itself with difficulty when placed ventral side up; when the disturbance is very pronounced the animal rotates around the longitudinal, transverse and dorso-ventral axes, nose dives, spirals, and swims on its back.

2. Allowing the factor of time to intervene, the animals which suffer profound disturbances of equilibrium—such as rotation around the axes, spirals and nose diving—regain most of their equilibrium within twenty-four hours. The animal swims normally in all planes, but if placed ventral side up, it swims on its back and rights itself with difficulty.

3. After sectioning the olfactory tracts or the optic nerves, the animal's equilibrium remains normal. Movement to the right or left around the dorso-ventral axis during the first hours following the sectioning of the optic nerves can not be accepted as disturbance of equilibrium because changing the direction of the animal changes the direction of its swimming.

4. Destruction of the labyrinths after sectioning of the olfactory tracts and optic nerves disturbs the animal's equilibrium similarly as the destruction of the labyrinths alone.

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THE STRUCTURE AND BEHAVIOR OF *ACTINOBOLUS VORAX* N. SP. (PROTOZOA, CILIATA).

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Actinobolus radians was first described briefly by Stein in 1867 and since then a number of observers have given descriptions of what they considered to be the same species. Thus, Entz ('82), Erlanger ('90), Calkins ('01), Moody ('12), Penard ('22) and Fauré-Fremiet ('24) have all described what they have called *Actinobolus radians*. But a review of their descriptions and illustrations makes it apparent that they were not all dealing with the same species. However the matter of the identity of the several species represented in the literature will not be dealt with here. It is sufficient at this time to point out that the species now to be described is different from any of those indicated in the available literature, and is therefore given a new name.

It is the purpose of the present paper to record some observations on the structure and behavior of this new species of *Actinobolus* which was found in the pond of the Botanical Gardens of the University of Pennsylvania. This ciliate appeared in considerable numbers in this pond during May, 1926. A number were fixed in various fixatives, chiefly Schaudinn's sublimate-alcohol-acetic and Bouin's picro-formol-acetic. Some were stained in toto with hæmalum and other stains, while others were sectioned and stained in a variety of ways but chiefly with Heidenhain's iron-alum hæmatoxylin.

Drawings represented by figures 1, 3 and 4, were made by Mr. R. M. Stabler to whom great credit is due for his care in executing them. I am also indebted to the Pennsylvania chapter of the Society of Sigma Xi for a grant of funds which made it possible to employ Mr. Stabler to make the drawings.

Actinobolus vorax is rather large, most specimens measuring between 100 μ and 200 μ in length with a width from ½ to ¾ as great. The form varies from an elongate oval to spheroid. The more elongate individuals are narrower and more tapering

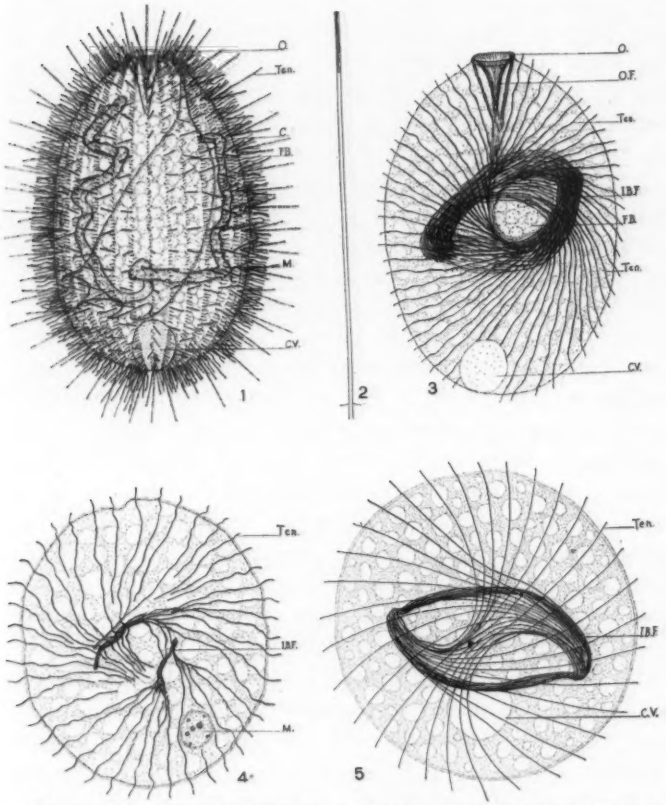

FIG. 1. Diagrammatic figure of *Actinobolus vorax* showing details of organization and the inclusion of an ingested *Anurea*. Tentacles partly extended. FIG. 2. Single tentacle, fully extended; toxicyst at distal end. FIG. 3. Side view showing inner extensions of tentacles and their association with the skein of internal fibrils. FIG. 4. Cross section of *A. vorax* showing tentacles and inner bundles of fibrils as seen in a thin section. FIG. 5. Whole animal viewed from posterior end; partly diagrammatic, showing bilateral arrangement of tentacles and their inner extensions connected with the skein.

EXPLANATION OF FIGURES.

All figures have been drawn at a magnification of 1000 and have been reduced about 3/5 in printing.

ABBREVIATIONS.

C.—cilia.
C. V.—contractile vacuole.
F. B.—food body.
I. B. F.—inner bundle of fibrils.
M.—macronucleus.
O.—oral opening.
O. F.—oral fibrils.
Ten.—tentacles.

anteriorly and more rounded posteriorly (Fig. 1). They are commonly a light yellowish brown in color. As in most other members of the family Enchelinidæ, the body is approximately radially symmetrical about its long axis with a mouth at the anterior pole and a cytopyge at the posterior pole.

Figure 1 indicates the general morphology of *Actinobolus vorax* with the tentacles only partially extended. This specimen is 110 microns long by 70 microns wide. The mouth at the more tapering anterior end does not usually protrude much beyond the general contour in the normal living animal but often protrudes slightly in fixed and stained animals, as indicated in the figure. Here it is about ten microns wide but the width varies in different specimens. The mouth is followed by a pharyngeal apparatus which is conical in shape, with the narrower end of the cone reaching into the body a distance of 20 to 25 microns. There are two sets of pharyngeal fibrils, an outer and an inner set. These fibrils do not seem to be of the nature of trichites. The inner group appears to be a thicker one, containing more fibrils and the outer set may include tentacles in its make-up as seen in some of the sections. The two sets of fibrils appear to converge at their outer extremities in what may be called the lip of the cytostome. In this lip there is a circular strand of more deeply staining material which may possibly be a sphincter for closing the mouth or may possibly be some part of a neuromotor system. In some stained specimens a second circular strand is seen a short distance outside the one just mentioned. Just how the two sets of longitudinal fibrils function is not known, but if they are contractile, the outer set might serve to open the mouth and the inner set serve to help close it or perform peristaltic movements which would aid in swallowing. While these animals were never seen to ingest anything except the rotifer, *Anurea,* stained specimens have, at times, revealed within them euglenoids, both encysted and not encysted, and several kinds of diatoms. In figure 1 an ingested *Anurea* is indicated.

At the posterior end one finds the large contractile vacuole (c.v.) which is usually subterminal (Fig. 3), and near it the terminal cytopyge which is not visible except during defecation and is not illustrated in the drawings. In many specimens there is a smaller

contractile vacuole on one side a little anterior to the middle of the body. It is not certain whether it is always present or may merely anticipate the next division. It has been noted more frequently in the more elongated specimens, but in stained animals it is seen in those in which there was no evident preparation for division.

The protoplasm is rather uniformly vacuolated or alveolar and contains the long, rope-like macronucleus which may be irregularly bent, is sometimes branched, and commonly makes a complete loop within the body, with the free ends at the anterior part of the animal (Fig. 1). Micronuclei were not definitely made out, although some small bodies were found which were tentatively identified as micronuclei.

The long cilia are arranged in from 30 to 60 longitudinal or slightly spiral rows. The tentacles are in the rows with the cilia, there being about thirty in each row. Thus the cilia are not arranged in groups about the bases of the tentacles, as described by Erlanger ('90), Calkins ('01), Moody ('12) and Penard ('22). The tentacles are fairly uniformly spaced through the mid-body region but less so toward the ends. There are usually from four to eight cilia between two adjacent tentacles in a row (Fig. 1).

The most interesting morphological character of *Actinobolus* consists in the series of tentacles which appear to be so unusual among the Ciliata although true tentacles are characteristic of the Suctoria. These tentacles are capable of being extended out in all directions to a distance as great as twice the diameter of the animal, yet may be withdrawn till they no longer protrude above the body surface. They are thus customarily withdrawn in actively swimming specimens but if a moving *Actinobolus* be followed for a time it will usually not be long till the tentacles gradually emerge from the surface and as they become longer and longer the swimming activities of the cilia gradually diminish till the animal comes to rest with the tentacles fully extended. After a few seconds or minutes the cilia become more active while the tentacles begin to shorten and soon *Actinobolus* begins swimming forward while the tentacles complete the process of re-entering the body.

The relatively long tentacles of this *Actinobolus* are uniform in diameter and at first appeared to be homogeneous throughout.

However, more careful study has revealed a highly refractive region within the outer portion for a distance of about ten to twelve microns from the tip (Fig. 2). This highly refractive rodlet is believed to be a trichocyst of the chemical type and therefor may be referred to by Visscher's ('23) term, toxicyst. The species of *Actinobolus* under consideration fed primarily upon the rotifer, *Anurea cochlearis* and the paralyzing effect of these toxicysts on *Anurea* is very evident whenever one of them comes into contact with the "forest of tentacles" presented by *Actinobolus*. One may therefore consider the tentacles as devices for extending the toxicysts out from the body, increasing thereby the area of possible contact between the ciliate and its prospective prey.

Each toxicyst is thus placed at the outer end of a relatively long and retractile stalk. But what becomes of the stalks when they are retracted into the body? What is the nature of these stalks? Are they composed of material that dissolves or changes to the sol state as they retreat inwardly, to be reformed when they emerge again, or are they permanent structures which must be accommodated within the animal's body? If the latter, how are they managed; what causes them to be withdrawn; what causes them to be extended? Have they extensile and contractile properties within themselves or do they wind up in some way as on a spindle or windlass when retracted and unwind when they are extended?

The inner structure of *Actinobolus vorax* as revealed by staining both entire animals and sections indicates that the stalks of the toxicysts are permanent structures that are accommodated within the animal when they are withdrawn.

In a specimen stained entire with hæmalum a group of fibrils was noticed in the interior of the animal arranged as shown in figure 3. Careful examination revealed that the tentacles could be traced inward from the surface of the body to join with an inner skein-like arrangement of fibrils. These findings were confirmed by the study of many other specimens both mounted entire and sectioned.

For example, Fig. 5 illustrates another individual as seen from the posterior end. This drawing is somewhat diagrammatic in that only enough of the tentacles and inner fibrils are shown to illustrate the general features of the arrangement. Fig. 4 repre-

sents a cross section outlined under the camera lucida. Apparently this section is viewed from the anterior end. Here, as in the two other drawings the inner extensions of the tentacles are seen to converge into two fibrillar bundles. The following statements will refer primarily to figure 3 since the entire system is more completely illustrated by this drawing. Here it will be seen that the inner extensions of the tentacles of the left side of the animal merge into the more posterior portion of the skein and converge at the right hand angle of the skein. From this angle a series of inner fibrils extend around and across the body by a somewhat anterior route to the left hand angle of the skein, being joined by the inner extensions of the tentacles from the right side of the body. From this left hand angle a bundle of fibrils extends around and joins to the right hand angle of the skein, thus completing the system.

There can be recognized four parts to this integrated system of fibrils: (*a*) a peripheral portion consisting of (1) the tentacles with their inner extensions from left side of the animal which converge to a point on the opposite side, and (2) the corresponding set of tentacles with their inner extensions from the right side of the animal; and (*b*) the anterior and the posterior groups of connecting fibrils which complete the skein. With such an arrangement it is difficult to avoid the impression that withdrawal of the tentacles would be accompanied by a winding up of the skein and extension of the tentacles would be accompanied by an unwinding movement. However, it is entirely possible that the extension and withdrawal of the tentacles may be due to extensile and contractile properties within the tentacles themselves.

A system of fibrils connecting with the cilia has not been made out but the inner ends of the pharyngeal sets of fibrils do seem to be connected with this system (Fig. 3).

The system of fibrils just described cannot be thought of as rigid in structure nor constant in position, although the general relationships are doubtless persistent. When one realizes that *Actinobolus vorax* will swallow such a relatively large object as a rotifer (*Anurea,* as in Fig. 1) and that room is sometimes made for two or three of these food bodies, it will be realized that this system of fibrils must accommodate itself to these relatively large

masses of food. In whole mounts of some specimens, the skein can be seen to be wrapped tightly around ingested Anureas.

In connection with the arrangement of the inner connecting fibrils and their accommodation to varying amounts of food, it may be noted that, as shown both in the sideview and polar views, the tentacles do not usually extend out in a strictly radial direction. When a living specimen is viewed from either pole, it can be seen that the tentacles extend out at such an angle as to indicate that their inner extensions pass somewhere between the center and the periphery of the body. In the living animals it is also noticeable that not all the tentacles extend out to the same distance from the body, some appearing to be shorter than others. It may be supposed that food bodies within the animals would interfere with the full extension of some of the tentacles.

Altogether, the evidence indicates that the tentacles are permanent structures, each bearing a toxicyst at its outer end, and also so connected and arranged within the animal to make an integrated system of fibrils, which presumably function in such a manner as to bring about the extension of the tentacles and their complete withdrawal.

Behavior.

The behavior of *Actinobolus vorax* is highly interesting as is that of all the species of *Actinobolus* so far described. If one chances upon one of these ciliates with its tentacles fully extended and then watches patiently it will usually not be long before the cilia become more active and the tentacles begin their retreat into the body. This withdrawal of the tentacles is gradual but takes only a few seconds. By the time the tentacles have been withdrawn about two thirds of their length, the cilia may be active enough to start the animal slowly on its way. Also one may see that the tentacles can be caused to wave about somewhat by the beating of the cilia, but they do not become bent in the process. As the ciliary activity increases, the animal gathers momentum and the tentacles complete their retraction so that they may be completely out of sight by the time the individual is going at full speed. In specimens that do not appear to be normal, the retraction of the tentacles may not be entirely completed and such

individuals may swim about with the tentacles partly protruding from the surface. As *A. vorax* swims forward it describes a spiral path and rotates on its long axis.

After a specimen has wandered about for a time, the tentacles begin to emerge again while the speed of progression gradually diminishes; and the longer the tentacles become, the slower is the swimming movement till the tentacles are fully extended and the individual is at rest, with its long axis horizontal. During this period of relative quiescence the cilia continue to move fitfully and sluggishly, but usually with the effective stroke toward the anterior end. After another period of quiescence, which may last for a matter of seconds or many minutes, the tentacles are again withdrawn and the animal swims away to come to rest in some other place. These alternate periods of swimming and quiescence are continued indefinitely and constitute the normal round of activity of this species.

If, now, while *A. vorax* is in its quiescent state with its tentacles extended out in all directions, a rotifer of the genus *Anurea* should swim against the tentacles, one would observe that the rotifer stops all movements as if completely paralyzed. The *Anurea* is then gradually drawn to the surface of the ciliate and is passed to the anterior end where it is brought in front of the mouth which expands sufficiently to engulf this relatively large food mass. All this indicates that the tips of the tentacles contain a paralyzing substance (in the toxicysts), that retraction of the tentacles draws the prey toward its captor and that either by the activity of the tentacles or the cilia, or possibly by both combined, the prey is carried forward to the mouth which opens and swallows the prey.

Watching the activities of *Actinobolus vorax* one is reminded of a fisherman who goes fishing with a copious supply of tackle. This fisherman wanders about till he sees a likely-looking place, then sets out all his lines and waits for the fish to "bite." If, after waiting till his patience is exhausted, he gets no "bites," he takes in all his tackle, goes in search of another promising spot and again puts out all his lines, repeating the process over and over again.

Actually, of course, *Actinobolus* does not *see* anything and makes no choice of a "fishing location." Under normal condi-

tions of the environment, the observed behavior constitutes its normal method of obtaining food; and since this behavior contributes to the welfare of the animal it may have the appearance of being purposeful. However, this behavior must be considered to be as automatic as any reflex action of a higher animal. If the environment becomes unfavorable, the behavior may be changed and the animal may swim indefinitely without coming to rest to "go fishing." Here again, its behavior contributes to the welfare of the animal, since continued swimming is more likely to bring it into a more favorable environment than would quiescence.

Discussion.

It is a little surprising that the internal structure of *Actinobolus* has not heretofore been described, but all the authors mentioned in the introduction, except Moody ('12) limited themselves to the study of animals in the living condition or else in temporary mounts after treatment with various agents.

The only internal structure mentioned by Stein ('67) was the nucleus. Entz ('82) stated that the tentacles could not be followed into the body but that when they are withdrawn they appear to vanish completely; and he found no trace of them after the use of reagents. Erlanger ('90) could make out the refractive trichocysts in the body after the tentacles were withdrawn, and could follow the inner part for some distance when the tentacles were partially extruded. Calkins ('01) does not mention the inner part of the tentacles but Moody ('12) traced them into the cortical region but not into the "endoplasm." Penard ('22) thought he could see the trichocysts imbedded in the cytoplasm when the tentacles were withdrawn, and Fauré-Fremiet ('24) reported and figured the tentacles as being visible for some distance into the body. However, none of these authors followed the tentacles inward to their connection with an internal system of fibrils, such as the present study has revealed.

Entz and Fauré-Fremiet described the cilia as arranged in rows as I have found them in *A. vorax*, while Erlanger, Calkins, Moody and Penard describe them as arranged in rows of clumps, each clump consisting of several cilia surrounding a tentacle. The animals with the cilia in clumps can scarcely be thought of as be-

longing to the same species as those with the cilia singly in rows. However, the animals described by Entz and by Fauré-Fremiet are so different in other respects from *A. vorax* that they can not be considered to be the same species.

It may also be noted that the feeding habits of the kinds of *Actinobolus* heretofore described are different in detail from those of *A. vorax*. Thus, Entz thought the kind described by him was capable of dissolving the cellulose walls of filamentous algæ and then devouring the cell contents. Calkins and Moody report that the *Actinobolus* studied by them "fished" always with its mouth downward and fed only on Halteria. *Actinobolus vorax* rests with the long axis horizontal and feeds primarily on *Anurea cochlearis* but mounted specimens have revealed within them other food bodies such as Euglenoids and diatoms. In its feeding activities, therefore, *A. vorax* appears to be as distinct from the other species as it is in morphological characters.

It may be pointed out that the system of fibrils here described has not been referred to as a neuromotor apparatus. It is doubtful if this term should be used for a set of fibrils that have no obvious connection with the locomotor organs, the cilia. However, there is a certain similarity between the system of fibrils in *Actinobolus vorax* and the system of fibrils described by Rees ('22) for *Paramecium*. One striking point of similarity is the bilaterality of the system, the fibers from each side of the body converging toward different centers. In his rather casual observations on the system of fibrils in *Paramecium*, the writer has come to believe that the distal ends of the fibrils connect with the trichocysts rather than with the cilia, although Rees thought that they connected with both sets of organelles. If their attachment should be found to be limited to the trichocysts, then the similarity between the system of fibrils of *Paramecium* and these inner extensions of the tentacles of *Actinobolus* would be fairly complete.

Summary.

Actinobolus vorax n. sp. is elongate oval to spheroid in contour and usually varies between 100 and 200 microns in length. There are between 30 and 60 longitudinal or slightly spiral rows of long cilia and about 30 tentacles distributed along each row. The cilia are not grouped about the tentacles.

The cytosome at the anterior pole of the body is followed by a pharyngeal apparatus which extends 20 to 25 microns into the body and consists of an inner and outer group of fibrils. These two groups of fibrils converge in the lip where there is a chromophylic circular strand which may be a sphincter or a part of a neuromotor system. There is a large subterminal contractile vacuole at the posterior end and usually a smaller lateral contractile vacuole a little in front of the middle of the body.

The protoplasm is rather uniformly vocuolated, although the central portion is sometimes more uniformly granular. The macronucleus is a long, irregular, rope-like strand, U-shaped, with the free ends in the anterior part of the body. Micronuclei were not definitely identified.

The tentacles are of uniform diameter throughout but contain a toxicyst at the distal end. They may be extended to a length equal to twice the diameter of the body and may be completely withdrawn into the body. They have inner extensions associated with an inner skein of fibrils. The inner extensions of the tentacles of the left side of the body converge to a point at the right side and vice versa. The internal skein is completed by connecting fibrils passing from the right hand center around the body to the left hand center and from the left hand center around to the right-hand center. It is suggested that retraction of the tentacles is accompanied by a winding up process and that extension is accompanied by an unwinding process. It is, on the other hand, possible that the tentacles have extensile and contractile properties within themselves.

In swimming, *Actinobolus vorax* turns on its long axis and describes a spiral path. While swimming the tentacles are withdrawn, but begin to emerge as the animal slows down and become fully extended when the animal comes to rest, as it regularly does with the long axis in a horizontal position. Alternate periods of swimming and quiescence constitute its normal behavior.

Actinobolus vorax feeds primarily on the rotifer, *Anurea cochlearis*. When one of these rotifers swims into the tentacles it stops all movement as if completely paralyzed. It is then drawn near to its captor and passed along to the mouth which opens and engulfs the prey. The same individual may ingest as many as

two or three of these large food bodies. In stained specimens of *A. vorax* euglenoids and diatoms have occasionally been seen.

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STUDIES ON THE STRUCTURE AND DEVELOPMENT OF CERTAIN CYNIPID GALLS.

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INTRODUCTION.

Our object in this paper is to present data from some macroscopical, histological, cytological, and physiological studies of certain Cynipid galls, and to correlate them with the work of previous investigators. In this way we have attempted to reach a

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general explanation of these observations that is more in harmony with the more recent concepts of physiology and morphology and enables us to include apparently discordant theories as parts of a larger concept of the mechanics and physiology of gall formation.

Material.

The material used for our investigations, with the exception of the galls of *Andricus futilis* form *futilis* O. S. from Sterlington, N. Y. in June, was collected from the trees of the Arnold Arboretum at Forest Hills, Boston, Massachusetts. Collections were made at intervals during May and June, beginning with the earliest determinable stages of the galls and ending with the emergence of the insect. The galls of *Amphibolips cookii* Gill. and *Disholcaspis globulus* Fitch used in the physiological experiments were collected during the first part of September.

Morphological and histological descriptions of the galls of the six Cynipids studied will be given in the following order: *Neuroterus batatus* form *bisexualis* Kins., *Neuroterus minutus* form *minutus* Bass., *Andricus petiolicola* Bass., *Andricus palustris* form *palustris* O. S., *Andricus futilis* form *futilis* O. S., and *Amphibolips confluens* Harris.

Technique.

For killing and fixing the gall tissues, Allen's modification of Bouin's solution was used and gave very good fixation in the insect tissues as well as in those of the plant. The very small galls were fixed whole, but the larger ones were cut into suitable portions to permit rapid and thorough penetration of the fixative. The material, after embedding in paraffin, was sectioned by the microtome to thicknesses of 6, 8, 10, and 12 microns. Hand sections of the living material were made for a few observations. Heidenhain's iron-alum hæmatoxylin together with orange "G" proved to be the most satisfactory combination of stains for general use, though safranin and gentian violet were also used in staining some of the sections. The presence of starch was detected by the use of a potassium iodide-iodine solution, confirmed by the use of polarized light. A solution of phloroglucine-hydrochloric acid was applied to sections to test for the presence of lignin.

Description of the Galls.

The earlier and in some instances the more recent descriptions of the structure of galls were published by those interested in taxonomy rather than by morphologists. We have noted these macroscopic conditions but our attention has been directed more particularly to the histology. For three of the galls, *Andricus petiolicola, Andricus palustris,* and *Amphibolips confluens,* Cook (1904) gave the first partial description of microscopical conditions, and Cosens (1912) added other observations on their anatomy.

Neuroterus batatus form *bisexualis* Kins.
Host: *Quercus alba.*

The gall of this insect is usually an irregularly elongate, polythalamous twig gall but it has also been reported on the petioles and mid-veins. Normal and deformed leaves may arise very close together due to the extremely short internodes of the dwarfed twig. A dense whitish pubescence covers the abnormal parts of the twig, but is not conspicuous on normal portions. The larval cavities are arranged in an irregular girdle about the twig outside the pericycle.

The epidermis of the gall, with the exception of the region about the entrance of the canal leading to each larval cavity, does not differ markedly from that of the normal twig. There are great numbers of very long trichomes clustered together about the entrance of each canal. The large number of larval cavities, each usually with a separate canal, makes it appear in a macroscopic view that the gall is covered by a dense, whitish pubescence. The wide zone of parenchyma between the epidermis and the tissue immediately under the influence of the larvæ is roughly divisable into two regions. One of these is 5 to 10 layers of cells in depth directly below the epidermis where the cell walls are thick; the other extends inward to the region of larval cavities where for the most part the cell walls are not thickened. Through this inner region of thin walled parenchyma scattered bands of cells with thick walls form an open network. Between the parenchyma proper, with cells quite normal in appearance, and the parenchyma that has differentiated itself into the zone designated as nutritive

there is a region in this gall, as there is before complete differentiation of the tissues has occurred in all the galls investigated, where slightly differentiated cells of meristematic nature show a transition to cells characteristic of the two adjoining zones. The innermost nutritive zone, sometimes in combination with the sclerenchyma when the latter is differentiated in the region of meristematic activity after this activity has ceased, is commonly spoken of as forming the larval chamber of the Cynipid gall. The cells of the nutritive zone, here a zone that varies from 8–12 layers in depth, are characterized by an increasing enrichment of protoplasm and by an increasing size of the nucleus and nucleolus as the larval cavity is approached. A few cell layers nearest to the larval cavity show a radial arrangement of the cells (Fig. 2). Cells of this kind are more abundant in the hemisphere of nutritive tissue toward the pith of the twig. This tissue nearest the larvæ always contains many cells with large nucleoli and a few with nuclei in various stages of fragmentation. A band of walls of recently emptied cells and remnants of other cells in advanced stages of dissolution border the larval cavity. It appears that the walls of these cells nearest the larva suffer gradual dissolution after the cell content is utilized. Between this band and the larva, there is usually what appears to be a mass of amorphous, jelly-like material which was observed in several cases to extend into an invagination on the ventral apical region of the larva. Toward the pith, there is a transition back from the nutritive cells to those of the parenchyma in contact with the vascular tissue of the twig. The outermost cells of the nutritive zone show an increase in the thickness of the cell walls as the larval cavity expands. A cessation of activity of the meristematic region intervening between this zone and the parenchyma proper is followed by a thickening of the cell walls in that intermediate region. In this gall, there is not, however, the pronounced lignification that commonly occurs in the mature galls of many species of Cynipids. This thick walled zone corresponds to the " protective zone " of some authors. In many other galls the completion of this process of lignification results in the formation of the stone cell type of sclerenchyma which forms a hard capsule about the larva.

It has already been noted that a canal leading to each of the

larval cavities is marked on the surface of the gall by an abundance of trichomes which arise from the epidermis of the surrounding region. This canal is usually clearly defined where it extends through the epidermis and parenchyma, but ends rather abruptly at the inner border of the latter zone. Through the nutritive zone the path is closed by parenchyma cells lacking the enriched protoplasm usually characteristic of the cells of that zone, and by a ribbon of dead and partially disintegrated cells. The clearly defined portion of the canal is lined with these partially disintegrated dead cells and is surrounded by two or three layers of parenchyma cells elongated parallel to the slightly curved canal.

When the tissues of the gall in various stages of development were tested with iodine, no stored starch was found. Under this treatment, a yellow to deep orange staining granular cytoplasmic content appears highly concentrated in the cells of the nutritive zone nearest the larva and gradually diminishes in quantity toward the parenchyma where it is no longer conspicuous.

Neuroterus minutus form *minutus* Bass.
Host: *Quercus alba.*

This is a polythalamous gall causing the leaves of the terminal buds to remain clustered to form an ovoid mass with a few crowded larval cavities in each of the small deformed leaves. The innermost leaves are not distinguishable but are fused into a mass of rudimentary leaf tissue crowded with the larvæ of the parasite. The galls are green and pubescent at the beginning of bud expansion, but mature rapidly and soon after the emergence of the insects become dry, shrivelled, and brown. These galls average 5–10 mm. in length and 5–8 mm. in diameter.

Cells varying in size and elongation form an epidermis with an irregular outline. Trichomes appear to arise most abundantly from the epidermis in the region of the entrance of the canals, as was found to be the case in *Neuroterus batatus;* but are neither so long nor so abundant as the trichomes on that gall. In the outermost leaves where some differentiation of tissue is noticeable, the cells of the parenchyma tend to radiate toward the larval cavity nearest them. The number of layers of parenchyma cells separating the cavities from the epidermis vary from three to as many as

fifteen. The innermost cells of this zone are radially elongated and adjoin the narrow sclerenchyma zone of small tangentially elongated cells with heavily lignified walls that abruptly separates it from the nutritive zone (Figs. 9 and 11). This zone has usually two layers of cells, though a depth of four layers is not uncommon, with the greatest lignification occurring on the inner tangential walls. Within the sclerenchyma, the cells are more or less oval with their greater extension radial toward the larva, and show the characteristic condition for nutritive cells as described in the preceding gall. The larval cavity is bordered, as is usual in the case of Cynipid galls, by a band of walls of recently emptied cells and remnants of others, with the adjacent cells in various stages of dissolution. Here also, as in the larval cavity of *Neuroterus batatus,* a mass of jelly-like material lies between the insect and the border of the nutritive zone. This intervening material is very similar in general appearance to the contents of the alimentary tract of the insect larva. It probably represents the secretions of the insect together with the dissolved cell contents of the adjoining nutritive zone, and the unicellular organisms that will be mentioned later. A distinctly uneven utilization of the nutritive zone is especially noticeable during the late larval stages of the insect (Fig. 11). In the inner part of the gall, the vascular tissues branch through the parenchyma and weave about among the crowded larval chambers. Here, as in the gall of *Neuroterus batatus,* the gall chambers are close to a comparatively large vascular system where there is a rapid movement of the nutritive material of the host.

The entrance of the canal is accompanied here also by an increase in trichome growth in the region immediately surrounding it. With the exception of the funnel-shaped opening extending for a short distance below the epidermis, the path of injury is marked only by the line of disintegrating cells which extend to the larval cavity itself. In the region of sclerenchyma, a conical plug of 5–10 layers of heavily lignified cells, with the point of the cone toward the end of the funnel-shaped opening, forms about the thin line of dead cells.

Sections of the galls containing larvæ, when stained with the iodine solution, showed starch to be present in the outer layers

of cells of the nutritive zone and in all the cells of the sclerenchyma zone. In the parenchyma, starch appeared in irregular patches; only a few grains occurring in the cells of this region, in contrast to the great quantity of grains in the other two zones nearer the larvæ. In the sections of galls containing pupæ, the starch had disappeared from the nutritive and sclerenchyma zones, but some of the cells of the parenchyma still contained a few grains. The cells of the nutritive zone, with the exception of the few outer layers in the cases of the younger galls, show no starch but contain an abundance of the granular content that stains yellowish or orange when treated with iodine, and which takes readily the hæmatoxylin stain. When the insect is ready to emerge from the gall, or has completed its demand for nourishment, the nutritive zone is usually exhausted and the starch also has now disappeared from the cells of the parenchyma.

Andricus petiolicola Bass.
Host: *Quercus alba.*

The gall is green, polythalamous, roughly club-shaped, or globose, and results from the swelling of the twig, petiole, or midrib of the leaf. Our observations were made from galls where both the twig and petiole were involved in the swelling. Galls involving these two plant parts were of extremely common occurrence. There is usually a short projection arising from some point on the gall with an opening into the interior. About the region of this opening there is an abundance of coarse trichomes. The larval cavities are more or less regularly arranged encircling the long axis of the gall, about equally distant from the center. The galls average about 8–10 mm. in diameter by 10–12 mm. in length.

With the exception of the region of abundant trichome growth about the opening of the canal, the epidermis shows no remarkable irregularities. The parenchyma zone is separable into two parts; the outer, immediately below the epidermis, is a broad region of thick walled cells; the inner portion lacks these thick walled cells and has vascular elements forming a more or less complete ring in its central region. In sections of very young galls, loops of small deeply staining cells of meristematic nature inter-

vene between this inner part of the parenchyma zone and the nutritive zone with its larger and richer cells (Figs. 1 and 3). In older galls, with the cessation of meristematic activity, secondary thickening occurs in the regions of these loops so that a region of stone cells (sclerenchyma) occupies approximately the same relative position as the earlier meristematic tissue. The cell walls of the sclerenchyma are in this instance generally uniformly thickened. The cells of the more central layers of this region show a greater thickness of cell walls, while toward the nutritive and parenchyma zones there are layers of cells with less heavily lignified walls; the outer layer of the nutritive zone and the adjoining parenchyma zone show a slight lignification of the cell walls. Relative to the larval cavity the two inner zones of tissues (nutritive and sclerenchyma, also the meristematic region while it is separate as such) show a notable eccentric disposition.

A common, large canal, presumably the path of the ovipositor, here reaches in from the epidermis and terminates in a more or less irregularly fusiform cavity from which smaller and shorter canals open toward the individual larval cavities. Cells resembling those of epidermal tissue were found lining this main canal only as far as the cavity. Many long trichomes arise from the epidermis about the opening of this canal while others appear to originate from the epidermoid cells lining the canal. There is a tendency for the epidermis to produce an increased number of trichomes wherever dead and disintegrating tissue occurs near the epidermis. Below the cells lining the common canal, 2–3 layers of much larger parenchyma cells appear; and these larger cells, below a border of injured and dead cells, mark the path of the shorter individual advance of each of the larvæ from the cavity at the end of the large canal into the plant tissues. About this cavity, between the paths of entrance to the larval cavities, regions of meristematic tissue occur in the very young galls in which many cells were observed in the process of division. This activity would appear to result from the stimulative influence of the disintegrating cells in the path of injury passing to the surrounding cells.

When sections of the very young galls (2–4 mm. in diameter) were tested with iodine or observed with the polariscope, no starch was observed. With the iodine treatment, the cells of the nutri-

tive zone showed an increasing enrichment of protoplasm as the larval cavity was approached. In older galls, where the larvæ were more advanced, starch was observed in the cells of the nutritive zone near the irregular surrounding sheath of sclerenchyma forming in the region previously occupied by the meristematic cells. The enrichment of the cytoplasmic content of the cells of the nutritive zone increases with the extension of the larval cavity and the resultant increasing proximity of the cells to the influence of the insect. There is a marked eccentricity of nutritive deposition in this gall (Fig. 1) with the tendency for the greater number of nutritive cells to coincide with the direction of greatest tension of the sap. The cells nearest the larva are marked by a process of dissolution resulting in a condition similar to that already described in the same region in the gall of *Neuroterus batatus*.

In the cells of the nutritive zone what appear to be unicellular organisms were observed to be very abundant. Fig. 7 shows some cells from this region containing such organisms, apparently bacteria. The presence of these organisms was checked in this and other galls by mounting and observing razor sections of the living tissues.

Andricus palustris form *palustris* O. S.

Hosts: *Quercus alba, Shumardii, palustris, ellipsoidalis, borealis maxima, Ludoviciana microcarpa.*

This form of *Andricus palustris* produces a spherical, monothalamous leaf gall that extends equally from the upper and lower surfaces. The gall occurs also quite commonly on the peduncles of the catkins. Until it reaches a size of 3–4 mm. it consists of a solid sphere of tissue surrounding the larva of the insect. With the cessation of meristematic activity and differentiation of the sclerenchyma tissue, the two inner zones of nutritive and sclerenchyma tissue separate from the outer parts of the gall. The small (1–2 mm.) spherical chamber composed of these two inner zones rolls freely about, in the mature gall, within the hollow sphere (8–12 mm. in diameter) resulting from the swelling and distention of the outer rind of parenchyma and epidermis. The old galls are green, mottled with whitish blotches that often assume a reddish color later. The very young galls show varying degrees of

pubescence and roughness of outline, but these features are lost as the gall matures.

The epidermis of the very young galls shows certain differences from that of the unaffected leaf, of which the increased length and number of trichomes is particularly noticeable. As has been already stated, there are slight differences in the cutin deposit and in the outline of the epidermis in different hosts. This variation applies to the parenchyma between the epidermis and the larval chamber where a variation in the number of cell layers appears, but there is no such observable variation in the inner two zones. Directly below the epidermis, the parenchyma has several layers of cells with thickened walls, while the remainder lacks the heavier walls and shows a gradual transition inward to the small, more or less isodiametrical cells of the region where cell division occurs. After growth has ceased in this narrow region of meristem, a lignification of the cell walls is effected which often extends in a lesser degree to the outer layer of nutritive cells. Thus it happens that a zone of sclerenchyma with ovoid, commonly binucleate cells, forms about the nutritive zone before the tissues separate. With the separation of the two outer from the two inner zones, the four layers of sclerenchyma cells that are generally present before the separation are then difficult to distinguish. It now appears as though there were only two layers with unequally thickened walls, the outer walls of the inner layer and the inner walls of the outer layer appearing much thicker than the others instead of the earlier condition in which the walls appeared equally lignified. With complete separation, a number of small undifferentiated parenchyma-like cells cling at intervals to the sclerenchyma and the inner rim of the parenchyma (Fig. 8).

In the nutritive zone the cells show the increase in size, the enrichment of protoplasm, the larger nuclei, and the increasingly prominent nucleoli characteristic of that zone. The increase in the size of the nucleolus (Fig. 17) is striking as the cells approach the region of contact with the larva. The cells bordering the larval cavity show various stages of dissolution. The depth of the nutritive zone is greatest at the time of separation; thereafter, it suffers increasing dissolution as the larva proceeds to make its demand for nourishment; finally, at the time the insect completes

the pupal stage, there is nothing left but a lining of a few incompletely utilized cells and remnants of cell walls bordering the sclerenchyma sheath. As in the other galls, a jelly-like material occurs between the larva and these tissues.

In our material the only indications of a canal or pathway leading to the larval cavity consisted of a band of dead tissue which is probably compressed by a healing process along the path of entrance of the insect into the plant. In much younger stages of the gall, Cosens has found a distinct canal with a lining continuous with the epidermis and bearing trichomes of the same kind as those appearing there.

Iodine preparations showed the starch to be restricted to the cells of the nutritive and sclerenchyma zones. As usual, the starch appears in increasing amounts in the cells not in immediate contact with the larva, while the yellow to orange staining granules increase in the reverse direction. Fig. 13*B* shows the starch distribution in a very young gall before separation of the zones has taken place. Fig. 12 shows under higher magnification the location of starch at the time of separation. In Figs. 13*A* and 13*B* it is possible to compare the amount of starch with the extension of the larval cavity and consequently increased sphere of parasitic influence, the greater amount of starch being present in the younger gall (Fig. 13*B*).

Andricus futilis form *futilis* O. S.
Host: *Quercus alba.*

This gall is polythalamous. It originates from the mesophyll of the leaf, and projects about equally from both the upper and lower surfaces of the blade. It resembles superficially the gall described by Cosens (1912) for *Andricus singularis* Bass. The lower projection of the gall is rather globular, but the one from the upper surface is like a flattened or suppressed cone in shape. Within the gall, two or three spheroidal larval chambers are suspended by fine strands of parenchymatous tissue. The galls have a diameter averaging about 4–5 mm.

The epidermal cells covering the gall differ from those of the normal leaf in being more flattened than cuboidal, and in having slightly thicker walls. Below the epidermis, there is a layer of

parenchyma cells not much larger than those of the epidermis but with thicker walls. Adjoining this layer is a region of larger, thick walled parenchyma cells which show a gradual change toward the interior until the inner region of parenchyma consists of more distended cells with thinner walls. It is from this inner region that the strands of cells are torn, radiate to the sclerenchyma, and hold the larval chambers in the center of the gall. In the mature gall the sclerenchyma is usually represented by two layers of cells with their outer walls much more heavily lignified than the inner ones, and conspicuously porous. The cells of the nutritive zone show conditions very similar to those of *Andricus palustris,* but show some variation in their arrangement about the larval cavity. In this gall, the nutritive zone assumes a slightly eccentric form with the greater depth of tissue opposite the region where the sclerenchyma zones of two or three larval chambers adjoin. In this region the cells are elongated and arranged radially with reference to the larva, while those nearer the place where the chambers join are smaller and more or less rhomboidal. Fig. 6 shows the relative position of the inner portions of part of a single chamber. The path of entrance of the insect into the tissue is similar to that in the preceding gall.

When sections of the galls were tested with iodine the starch was found to be confined to the sclerenchyma and nutritive zones. The innermost layers of the nutritive zone showed no starch, but contained the usual abundance of granular protoplasm.

Amphibolips conflens Harris.
Hosts: *Quercus velutina, Q. coccinea.*

Amphibolips confluens produces a large, monothalamous, globular gall that arises from the petiole or midrib of the leaf, and is filled with a succulent, white, spongy, fibrous tissue. In the center of this spongy tissue, there is embedded a comparatively large, thick, woody capsule which incloses the nutritive zone and the parasite. The galls are green and pubescent when they are young, but the green color changes to a light, lustrous brown, and the pubescence disappears as the gall reaches maturity. There is usually a noticeable protrusion from some place around the periphery marking the entrance of the canal leading to the larval cavity.

When the gall has a diameter of 3–4 mm., the epidermis has numerous clusters of trichomes and the epidermal cells are larger and thicker walled than those of the normal leaf tissues. Immediately below the epidermis, there is a region of parenchyma cells about six layers in depth showing a transition from thick walled cells with small vacuoles to larger, thinner walled cells with increasingly prominent vacuoles that adjoin the elongate parenchyma cells which are arranged in rows radiating toward the center of the gall. At this stage of development, separation of the rows of cells into strands has already begun in the region of elongation. Toward the center of the gall there is a transition from the elongate, radiating cells to smaller and more spherical ones with many minute vacuoles, a granular cytoplasmic content, and often two nuclei. These cells in turn show a transition to those of the nutritive zone which are much larger and assume a radial arrangement (Fig. 4). The vascular tissue radiates abundantly through the region of small cells outside the nutritive zone and in the inner region of the parenchyma; but appears to turn and run parallel to the periphery in the outer part of the parenchyma, and is more abundant at the region of attachment of the gall. The isodiametric, or slightly ovoid cells observed at this stage surrounding the nutritive zone, represent the previously active meristematic tissue. These cells from now on have their walls increasingly lignified, until in the later stages they form the sclerenchyma zone inclosing the parasite in a woody capsule.

The opening of the canal into the larval cavity is marked by a small delta from which a band of disintegrating cells and a ribbon of cell remnants extend outward to the epidermis. About this band of tissue, there are large cells, often polynuclear (Fig. 26), which are elongated parallel to the path of the canal. From these cells, there is a transition back to the type found in the zone through which the canal passes. A conical protrusion of the epidermis and outer layers of the parenchyma marks the opening of the canal to the exterior. A conical depression lined with a heavy band of dead tissue surrounded by thick walled parenchyma cells occurs within the conical protrusion. The epidermis does not appear to line the entrance of the canal, but ceases at the edges of the opening. The trichomes seem to be most abundant near the

entrance of the canal, but appear never to arise from the interior of the canal.

Starch is present as small grains in the cells of the outer layers of the nutritive zone and in those of the layers of isodiametric cells. The granular cytoplasmic content of the cells is greater nearer the larval cavity and less in the cells more distant, as in the preceding galls.

When the gall has reached a diameter of 10 mm., the epidermis has stretched until the cells are long and narrow, and the trichomes are lost. The cells of the parenchyma zone have increased greatly in size with an enlargement of the vacuoles. The separation of the inner region of parenchyma into strands has progressed until the two inner zones which surround the larva are suspended by them from the outer thick walled region of parenchyma, together with the radiating fibro-vascular bundles. The nutritive zone shows greater dissolution in the innermost layers. The zone of adjoining small cells is narrower, and occasionally some of them develop a form more characteristic of the nutritive zone on the one side and of the parenchyma on the other, their walls have become slightly lignified, and the elements of vascular tissue are now more distinct (Fig. 5). This same photograph shows that there has been a marked increase in the quantity of starch in the cells of the outer layers of the nutritive zone and in those which lead toward the region of radiating parenchyma.

In the late stages of the gall, the strands holding the inner two zones to the outer zones become more attenuated; the region adjoining the nutritive zone is now prominent and its 10–15 layers of cells with heavily lignified walls form a woody capsule of stone cell sclerenchyma. Nearest the nutritive zone, the walls of these cells appear to be slightly more lignified away from the larva, while the remainder appear to have uniformly lignified walls. The large quantity of starch observed in the young galls has entirely disappeared and only the orange staining granular content appears in the remaining nutritive cells.

Enzymes in the Galls.

The morphology of the galls indicates the presence of certain definite enzymes that act in a centrifugal direction from the larval

cavity. In the course of the larval development, the abundant accumulation of starch in the nutritive zone of the gall gradually disappears, first from the inner, then from the outer layers; that is, a gradual hydrolyzation of the starch occurs in a centrifugal direction. Hand sections from the galls of *Disholcaspis globulus* treated with the potassium iodide-iodine solution showed a graduation of the intensity of the blue color of the starch grains. The grains in the outermost layers of the nutritive zone stain a very deep blue, but the intensity of this color decreases in the direction of the larva until the starch grains of the innermost layers of the zone take no stain. These non-staining grains are apparently no longer real starch grains but are merely the membranes of the grains whose starch has been already hydrolyzed. We have naturally inferred that diastase must be present in the very innermost layers of the nutritive zone, and similarly protease, phytoprotease, and amidase to effect the destruction of the contents of the cells surrounding the larval cavity; hadromase to bring about the reduction of the sclerenchyma. Finally, we must mention the presence of cytase to effect the hydrolysis of the cell walls which takes place where their contents are in very advanced stages of dissolution.

The presence of diastase was confirmed by physiological experiments. Well developed galls produced by *Amphibolips confluens* on *Quercus velutina* were collected and carefully opened without injuring the larvæ. The larvæ were washed with distilled water and the pH of the solution obtained was tested by the Clark (1920) color indicators. In the same manner, the larvæ of a parasite of *confluens* were washed and the solution tested. Both of these solutions had a pH of 7.0. Extracts from the few inner layers of the nutritive zone were prepared with distilled water and found to have a pH of 6.7. The solution obtained by extraction from the parenchyma tissue of the gall with distilled water was found to have a pH of 4.6, while extracts of the normal leaf tissues of this host had a pH of 4.0 to 4.6. These data are tabulated in table 1 together with those for the activity of these solutions and of saliva on potato starch.

TABLE I.

No.	Solution.	pH.	Change Effected in Starch.
1	Solution obtained by washing larvae of *Amphibolips confluens* with distilled water.	7.0	No morphological change in the starch grains.
2	Solution obtained by washing the parasitic larvae with distilled water.	7.0	No morphological change in the starch grains.
3	Extraction from the inner layers of the nutritive zone tissue with distilled water.	6.7	Most of the starch grains shrunken and broken; emptied of starch content.
4	Extraction from the gall parenchyma tissue with distilled water.	4.9	No morphological change in the starch grains.
5	Extraction of normal leaf tissue with distilled water.	4.0 to 4.6	No morphological change in the starch grains.
6	Saliva.	7.3	A few of the starch grains broken; solution is less active than No. 3

The starch was obtained from potatoes and was washed thoroughly four times in distilled water before being treated with the various solutions of extracts. The presence of a hydrolytic enzyme was observed only in the third combination of table 1, that is, when the starch is subjected to the activity of the extract from the inner layers of the nutritive zone (Fig. 16). The activity of this extract is much more effective in hydrolyzing starch than is saliva. Starch grains stained by iodine solution destained gradually after adding an extraction of the nutritive zone in distilled water. In reality, no destaining of the iodine stained starch grains takes place, but a hydrolyzation of the starch is effected, so that, in place of starch grains, sugar and empty grain membranes result, neither one of these substances staining when treated with iodine. With hydrolysis, the starch grains are readily broken and destroyed (Fig. 16). Cosens (1912) has carried out experiments showing hydrolysis of starch to sugar under the influence of the larvæ. As yet, however, no experiments answer the question of whether all the starch is hydrolyzed directly by the insect secretions, or whether there are other sources for the production of agents of hydrolysis in Cynipid galls. This question appears to be reasonable, since unicellular organisms, also capable of producing enzymes, are abundant between the insect and the plant, appearing in the jelly-like material already noted in permanent preparations,

and in the inner layers of cells of the nutritive zone. The questions also arise: To what extent are the insect secretions responsible for the formation of the gall and for the gradual destruction of the plant tissues about the larval cavity? And to what extent are the activities of the unicellular organisms found in the gall responsible for these phenomena? The answer to such questions must await the performance of extensive experiments dealing with this aspect of gall formation.

The gradual decrease of the pH value in the galls in a centrifugal direction from the larvæ is further shown in tests made on the tissues of the galls produced by *Amphibolips cookii* Gill. on *Quercus alba* (Table 2). The data from these two tables (Tables 1 and 2) suggest an ion exchange between the larval and plant tissues.

TABLE II.

No.	Solution.	pH.
1	Extraction of larval tissue of *Amphibolips cookii* Gill. with distilled water.	7.0
2	Extraction of the nutritive zone tissue of the gall with distilled water.	6.7
3	Same as No. 2 but tested twelve hours after extraction.	6.7
4	Extraction of the parenchyma strands of the gall with distilled water.	5.6

The reduction in extension and number of layers of the sclerenchyma zone just outside the nutritive zone is indicative of the presence of hadromase. In some cases, as in the gall of *Andricus palustris*, the apparent reduction of the number of layers of cells in the sclerenchyma zone may be due rather to the mechanical compression than to the hadromatic activity. This question will be discussed more thoroughly later.

SOMATIC CELL DIVISION IN PLANT AND LARVAL TISSUES DURING THE FORMATION OF THE GALLS.

The effect of mechanical and chemical stimuli on the living tissues during gall formation appears to be similar to that in plant and animal tumors, graft unions, bacterial nodules, and certain calluses.

The plant cells, in all of the galls studied, which are in immediate contact with the insect or are exposed to direct influence

of the larval secretions and of unicellular organisms do not divide. The lysins first break down the nuclear membrane of the plant cells (Fig. 10) and then gradually dissolve the entire cellular content. The nuclei of the cells which are under intensive attack by the lysins resemble the nuclei of the cells of the nodules of *Phaseolus vulgaris* which contain nodule bacteria (Fig. 15).

The entire nutritive zone, being under direct and powerful influence of the larval secretions, is characterized by swollen cells and by the absence of cell division. The cells outside the nutritive zone, namely those of the sclerenchyma zone, very often contain two and sometimes three or more nuclei (Fig. 19). Binuclear and often multinuclear cells appear also in the few layers of parenchyma cells adjoining the sclerenchyma zone (Fig. 22). The occurrence of multinuclear cells appears to be a general phenomenon when plant and animal cells are exposed to certain very active stimuli. Such is the case in animal tumors (cancers) and plant tumors (nodules caused by bacteria, callus tissues, and gall tissues). The opinion that an increase in the number of nuclei in the cells of such tissues is brought about by amitotic division appears very frequently in the literature. Our figures show what may be considered to be a common occurrence in such cases; namely, that the chromosomes divide mitotically but are highly inactivated by foreign stimuli so that their separation and removal from the equatorial plate is delayed (Fig. 21). The two chromosome sets, lying very close together as a result of this delay, form two nuclei between which no cell wall forms. The inactivation of the chromosome separation by the larval influence is well shown in the cells of the gall produced by *Neuroterus batatus bisexualis* on *Quercus alba* (Fig. 23). When the spindle is perpendicular to the direction of the activity of the stimuli, larval secretion, etc., as in Fig. 23, the inactivation of the chromosome separation is usually greater from the side toward the larval cavity, the source of stimuli, and causes irregularity of the somatic cell division (Figs. 23 and 25 *A*).

The somatic chromosomes of the cells which are in the region of the active influence of the larval secretion—that is, in the meristematic region of the gall—appear round or ovoid in permanent preparations. Conformations such as these are charac-

teristic of the pollen mother cells and embryo sac mother cells, the chromosomes here also being often found to lag on the spindle. At a greater distance from the larva, where the larval secretions are apparently inactivated by the protective substances of the plant, the somatic chromosomes are slender, prolongated, and U, J, or I shaped (Fig. 25 *B*)—the usual conformation of normal somatic chromosomes. In *Quercus alba,* 22 somatic chromosomes were counted in most cases; but in a few plates, 24 chromosomes were observed. Apparently 2 of the chromosomes have a tendency to fragment.

Around the canal of the gall produced by *Amphibolips confluens* on *Quercus velutina,* cells with several nuclei were observed. In some of these cells, the chromatin to all appearances divides continuously without separation of the chromosomes and forms large chromatin masses usually imperfectly organized into nuclei of different sizes (Fig. 26). A similar phenomenon was observed by Němec (1924) in the galls of *Eriophyes padi* Nal. on *Prunus spinosa* L. The substances which are products of the disintegration of the dead cells along the canal are evidently the agents responsible for the abnormality of the cells surrounding it.

Multinuclear cells (Fig. 24*A*) were also observed in the larval tissues. Certain cells, especially those around the alimentary tract, are very large—often 200 times larger than some of the cells of other parts of the body—and have exceedingly large nuclei (Figs. 14 and 18). These conditions are evidently due to the activity of foreign substances on the larval cells and apparently result from numerous chromosome divisions without any accompanying cell division. This phenomenon appears to be very common in other larval tissues. Cells of *Andricus palustris palustris* with 10 chromosomes (Fig. 20), with 20 chromosomes (Fig. 24*B*), with about 40 chromosomes, and sometimes cells with many more chromosomes were observed. In many instances, irregularities in somatic cell division, such as lagging of chromosomes (Fig. 27), were observed in the larval tissues. The appearance of polyploid cells and irregular mitoses in the larval tissues would seem to indicate the presence of active foreign substances. These substances are produced by derivatives of plant tissues and of disintegrated plant cells, unicellular organisms, and by phenomena

connected with the metamorphosis in the insect itself. The mutual reaction between the plant and the larva, in the presence of the unicellular organisms, is apparently expressed similarly in both plant and larval tissues. Thus, cells of Figs. 10 and 26 from the plant correspond to cells of Figs. 14 and 18 from the animal, Fig. 22 corresponding to Fig. 24*A*, and Fig. 23 corresponding to Fig. 27.

Generalization of the Data and Discussion.

Malpighi (1686), the earliest writer on gall formation, postulated an introduction of a substance into the plant tissue which caused gall development ("vitrioli enim portio, quæ in Quercubus luxuriat, infuse terebræ ichore, turgentiam concipit"). Lucaze-Duthiers (1853) divided the problem into two parts, the definition of a gall and the cause of a gall. The latter phase he divided again into three parts, these concern: (1) the wound, (2) the action following the wound, and (3) a "special liquid" which is deposited in the region where the gall develops. The first two points he recognized as secondary in importance; the real cause was thought to be in the third point, the "venin" deposited by the insect with the eggs into the plant tissue. A similar view was expressed by Prilleaux (1876). He assumed that the cause of gall formation was mainly ". . . l'irritation spécifique que accompagne le dépot de l'œuf et que cause probablement une sorte de venin que l'insecte verse dans la plaie."

Adler (1881) supposed that the biting of the larva was the responsible factor in gall production: "In den Augenblick nun, wo die Eihaut durchcrochen hat und zum ersten Male mit den feinen Kiefern die nächstgelegenen Zellen verwundet, beginnt eine rapide Zellen-Wucherung." Two years later, Adler's conception was questioned by Beyerinck (1883): "Einige Autoren (meaning Adler) haben in dem Nagen der Gallenlarve einem Reiz sehen wollen, welcher, noch ihrer Ansicht die pflanzliche Gewebe affizieren, möglicherweise zur Wucherung bringen konnte. Freilich besitzen die Cynipidenlarven schon dann, wenn dieselben noch als wollständig kugelförmige Thiere innerhalb der Eischale eingeschlossen sind, feine Chitinkiefer, allein, zu dieser Zeit, wenn von einem Zernagen der pflanzlichen Zellen natürlich kein Reden sein kann, ist das Wachstum des Gallplastems schon in vollen Flusse."

The active substances for the gall formation, according to Beyerinck, are the "Wuchsenzyme," that is, diffusible excretions at first from the egg and later from the larva.

The interpretations of Adler and of Beyerinck on gall production were accepted by the recent investigators of galls, very often with only slight modifications. We may cite Weidel (1911), Küster (1911), Cosens (1912), etc. Since a full treatment of hypotheses concerning gall development was given by Magnus (1914), we will not go into detail here. Studies on plant and animal tumors, on graft unions, and on species hybrids, and the accumulation of new data on the physiology of development and immunology correlated with the observations of previous investigators and our own observations given in the present paper offer a new basis for the discussion of gall development and its causal interpretation.

In the course of our discussion, the first general question is how a gall begins. It is better to orient ourselves with a brief description of oviposition. Beyerinck (1883) points out three possible methods for the deposition of the Cynipid egg: . . .

"entweder schiebt das Thier die Legeröhre zwischen die Pflanzentheile, ohne diese und das gallbildende Gewebe zu verwunden; oder es erzeugt zwar eine Verwundung, um das Ei jedoch an eine vollständig unversehrte Stelle zu bringen oder endlich ist legt das Ei in eine in unmittelbarer Nähe des gallbildenden Gewebes angebrachte Öffnung. Auch fur diesen Fall werde ich zeigen, dass die Gallbildung durch die Verwundung nicht beinflusst wird."

The first real causal interpretation of the beginning of gall formation dates from Beyerinck, older investigators having given a rather teleological explanation of its development. His thorough investigations (1883) demonstrated that:

"Wenn die Eier an die äussere Oberfläche der Organe der Nährpflanze niedergelegt werden, ist es klar, das Plastemwall, welcher sich ringsum den Larvenkörper erhebt und diesen zuletzt gänzlich vergräbt, überall von dem ursprunglichen Hautgewebe der Pflanze bekleidet ist, und dass demzufolge auch die Gewebe des Kammerloches und der Larven Kammer aus der Epidermis der Nährpflanze entstehen. Die Gallen welche sich auf dieser

Weise entwickeln, und deren Narbe,—das heisst die Stelle wo sich der ursprüngliche Plastemwall nach der vollendeten Umwallung geschlossen hat,—irgend auf der freien Gallenoberfläche vorkommen muss, kann man 'Gallen mit äusseren Verschlusse' nennen. Werden dagegen die Eier innerhalb der Gewebe der Nährpflanze gelgt, so schliesst das Plastem sich in der Weise, dass die Narbe vollständig verborgen im Innern des betreffenden Organes zu liegen kommt, und solche Gallen liessen sich unter den Namen 'Gallen mit innerem Verschlusse'" (p. 182). In the first case, the larva . . . "im Gallplastem vergraben ist. Dem Frasse an und für sich, kann man demnach keine Bedeutung bei der Gallbildung anerkennen" (p. 180).

Just the opposite opinion was defended by Weidel (1911).

"Die in der Eihaut noch vollständig eingesclossene Larve durchbricht diese an einer Stelle und senkt in die Epidermis des Blattes ein Organ ein, durch das die Cuticula durchbrochen und das Pflanzliche Gewebe verletzt wird, ganz analog der von Magnus festgestellten Verletzung bei der Rose, nur dass sie dort schon bei der Eiablage stattfindet" (p. 287). He assumes . . . "dass die Kiefer seien, die in die pflanzliche Epidermis eingesenkt werden."

On this point, we entirely agree with Cosens (1912) that, though the excellent work of Weidel cannot be questioned concerning this particular gall, it is not necessary to assume that this is the only method by which a larval cavity is formed.

In each method of oviposition, whether accompanied by wounding or not, the egg has on its surface substances which irritate the plant cells as they are foreign for the plant tissue. Many investigators have observed an egg excretion, a fluid between the egg and the plant cells. This is the stage when the unicellular organisms (generally bacteria) start their activity between the egg and plant tissue, no matter whether the plant tissue has suffered any mechanical injury or not. The substances around the egg—the egg excretions, the irritable substances produced by the bacteria and probably the other unicellular organisms, and the wound hormones (Haberlandt, 1922), when the oviposition is accompanied by an injury to the plant tissue—are the substances which give

the first impulse for the development of a gall. The wound hormones produced in the injured tissues must certainly be included among the substances which give the first impulse for the gall formation. Our conception of these wound hormones will be given more fully later.

The next question is how the irritable substances act to cause the development of a gall. This question can be easily answered when one considers that the single cell, as a member of the whole tissue, acts and reacts very similarly to an organism as a whole. When the latter, the organism, is exposed to a very insignificant stimulus, it does not react at all or but very slightly. With the increasing of the quantity of the stimulus, the reactivity of the organism also increases; this is expressed by increased metabolism, more rapid growth, *i.e.*, by more intensive cell division. When the stimulus increases quantitatively, this growth does not continue at an increased rate. There is an optimal point beyond which a quantitative increase of the stimulus causes retardation of growth until an entire inhibition of cell division is effected. If the quantity of the stimulus is increased beyond this point, the organism dies. When the stimulus is localized, the same sequence is true for a single cell, for a group of cells, or for a tissue. In gall formation, there is a series of stimuli as described above, which are localized at a definite place, *i.e.*, around the egg and later around the larva, acting in a centrifugal direction from this center of irritation.

Shortly after oviposition, the response to the stimuli is limited to a very small area around the egg. The first impulse is a stimulation of cell division below or around the egg. With increasing stimuli, the responsive area increases, but, at the same time, a gradation in the intensity of the stimuli occurs. The stimuli are too strong for the cells with which they are in immediate contact and later they become too strong for the cells a few layers further away. These cells do not divide; they are inactivated and swollen; some of them undergo dissolution because of the lytic activity of the foreign substances. Beyond the highly inhibited cells, there is a region where no very intensive cell division occurs because the quantity of the irritable substances is still in excess of the optimum. Then comes the zone with the greatest frequency of cell

division; this is under the activity of the optimum quantity of the stimulative substances. Still beyond this optimum region, the rate of cell division is relatively low due to an insufficient amount of inductive substances, and to all appearances parasitic influence does not extend to the more distant regions.

It is pertinent to mention two recent conceptions for the cause of cell expansion and division in normally growing tissues—the one, the "growth substances" of Went (1928); the other, the "mitogenic rays" of Gurwitsch and his students (1926, 1927, etc.)—and to point out the significance of the same cause for cell division in the plant galls caused by Cynipids. There is a high degree of probability that in the near future the two terms just mentioned will be found to be two components of the growth phenomenon. Generalizing from the experimental data of Gurwitsch and others, one arrives at the conclusion that growth *i.e.*, cell division, is induced by mitogenic rays. Magrou and Magrou (1927) succeeded in inducing cell division in onion root tips by cultures of *B. tumefaciens* located at certain distances from the root tips, as Baron (1928) did later with many other bacteria, and concluded that the tumors were induced by the mitogenic rays emanating from the bacteria. It would seem at first glance that their supposition would be plausible for the tumors and applicable to the development of Cynipid galls where the growing insect and a multitude of unicellular organisms, including bacteria, are present in plant tissues. When we consider the chemical phenomena resulting from various substances and enzymes introduced by the parasites and the reaction of the plant to these diffusing substances, together with the necrosis of the plant tissues resulting from the activity of the parasite, any possible part played by the mitogenic rays emanating from the parasites in the development of the Cynipid galls appears very insignificant in comparison with the other physiological factors concerned.

An opinion about the activity of the "Wundhormone" and "Necrohormone" which were studied by Haberlandt (1922) may be expressed here in connection with our studies. As Haberlandt (1922) and others have shown, when cells are wounded or die from some unknown internal cause, intensive cell division occurs around the injured or dead cells. But we must ask whether there

is any necessity accepting a special terminology as Haberlandt proposed, when we do not know whether definite substances of the same nature as the hormones produced by the thyroid, hypophysis, and other glands of internal secretion that induce cell division in animals, are always present at the injured place. When a cell dies from mechanical or chemical injury, its content undergoes disintegration; the cell and body specific substances are destroyed and change into other substances foreign for the surrounding tissues no matter of what kind they are. These disintegration products irritate the surrounding tissue and stimulate cell division.

Cell division in gall development can be attributed partly to the disintegration products of the cells injured: (1) mechanically during the first period of development when a wounding by oviposition occurs, and later when wounding occurs by chewing if the larva is one which feeds in this way; (2) chemically by the action of the egg or larval secretions and the toxic substances produced by the unicellular organisms. Hence, we can not agree with Beyerinck (1883): "Dem Frasse an und für sich kann man demnach keine Bedeutung bei der Gallbildung anerkennen" (p. 180). At the same time, we can not agree with Weidel's objections (1911) against Beyerinck's interpretations of the "Umwallung," "Sinken," and "Vergraben" when he questions: "Wie kommt es, dass an der Stelle, wo das von der Larve abgesonderte Enzym am stärksten wirken muss, keine Vergrösserung der Zellen stattfinden soll, sondern nur in einiger Entfernung? Was wird aus Epidermis unmittelbar unter dem Ei? Aus Beyerinck's Figuren muss man annehmen, dass sie in Nährgewebe umgewandelt wird, da sie die Larve unmittelbar beruhrt. Wie kommt das "Sinken" oder "Vergraben" zustande, Vorgänge, für die ihn seine Erklarungen selbst nicht befrieddigen?" (p. 290).

If Beyerinck (1883) could have forseen the later developments of physiology and immunology, perhaps, he would have interpreted his observations in another light, and if Weidel (1911) could have had at his disposal the still later discoveries in the same fields, he would have seen that his observations and those of Beyerinck could be correlated. Weidel believed that the larval chamber was formed by a dissolution of the subjacent tissue not from a process of "Umwallung" such as Beyerinck supposed.

When oviposition occurs on the surface of the plant, the process of enclosing the eggs and later of the larvæ occurs by "Umwallung," "Sinken," and "Vergraben," and also by "Lösungsvorgang" (Weidel), terms which we will discuss in a moment. The metabolic processes of the cells exposed to the strongest activity of the stimuli differ from those of the ones further removed. These cells do not divide; they are too highly inactivated. They only enlarge because of the very abundant sap supply, which represents a part of the reaction of the plant tissue against the irritable substances. The lytic substances, the enzymes, attack the cells in immediate contact with the parasite and dissolve them. This process of dissolution occurs continuously and as a result the egg, and then the larva, enter gradually into the plant tissues. This corresponds to Weidel's "Lösungsvorgang" and Beyerinck's "Sinken." If any chewing occurs on the part of the insect, the sinking is accelerated.

Beyond the zone of inactivated cells subjected to progressive dissolution, where no cell division occurs, is the zone exposed to the optimum stimulation in which there is a very intensive cell division. The latter zone forms in a position approximately concentric to the former, *i.e.*, like an hemispheroid. The physiological processes are expressed shortly in mechanical deformations and transformations. The hemispheroidal area with intensive cell division tends continuously to complete a spheroidal form. This process corresponds to Beyerinck's observation on "Umwallung." When the complete spheroid develops from the earlier hemispheroid as a consequence of the intensive cell division in the zone with optimum irritation for cell division, the irritable and destructive substances act symetrically in all directions from the center of irritation, *i.e.*, from the larval cavity. This is the stage Beyerinck calls "Vergraben." His terminology is perhaps too rough for these processes, if one treats them in the light of the physiology of development; yet his observations come into accord with those made by Weidel (1911), Cosens (1912), Magnus (1914), etc. only on the basis of the above interpretation. Serious discrepancies which occur in the cecidological literature are not in the observations but in the interpretation of the facts observed. We hope that our interpretation gives a causal explanation of these processes and their consequent morphological manifestation.

With the preceding discussion we answered the question "why and how does a Cynipid gall begin?" Next, one must ask when, why, and how does the growth of the gall cease? In order to answer these questions, we have to consider the ability of the plant tissue to react against foreign substances. Here, another question arises: Is the plant organism or plant tissue able to produce antibodies against foreign substances as the animal organism or animal tissue is able to do? It has already been shown by the senior author (Kostoff, 1928) that plant tissues can acquire immunity against certain antigenic agents of other species by some type of antibody production. Normal precipitins occur in leaf and stem extracts and the precipitin potency of certain species and genera is increased after grafting; moreover, in certain combinations whose extract shows no precipitin reaction before grafting, the capacity to produce precipitins is acquired during the growth of the graft unions. Considering these facts, one can give a plausible theoretical interpretation of gall formation and answer the above question.

The larval secretion, the products of the unicellular organisms around the larvæ, the disintegration products from the destroyed cells of the innermost layer of the nutritive zone, and the disintegration products of the cells destroyed by the larval chewing, if any occurs, represent the stimulative and antigenic agents that penetrate into the plant tissue. At the beginning of the gall formation their amount is, relatively speaking, very limited, but their quantity increases with the time of larval growth. At first, the penetration of these stimulative substances is prevented, not only by mechanical retardations, but to a certain extent by the normal unspecific protective substances (antibodies) which are present in the plant. Later, when the quantities of the stimulative substances increase enormously, they effect a greater area of the surrounding tissue in which there is an optimum region of intensive cell division. The affected regions, however, do not spread proportionately to the amount of irritable substances produced. There is a period of time when the quantity of the latter continuously increases without any further increase of the affected area. At this time the plant tissue begins to produce a sufficient amount of protective substances induced by the penetration of foreign sub-

stances. During the course of larval development, the amount of these foreign substances in the plant tissues increases; at the same time, however, the production of the protective substances also increases. There is a time when the quantity of the latter increases to such an extent that it neutralizes the effect of the foreign substances, even in the region where cell division is most intensive; in other words, *the protective substances eliminate the stimulus for the cell division and the growth of the gall stops.* This defense extends to the highly inactivated nutritive zone. Outside this zone, in a region of a varying thickness in different galls, the neutralization between foreign and protective substances occurs. This neutralization zone is the one marked by the sclerenchyma in the galls described.

We have given the general outline of gall formation, and may now inquire whether all the individual morphological and physiological observations can be arranged under the preceding outline. This question may be subdivided into three parts for greater convenience of treatment: (1) observations on the nutritive zone; (2) observations on the sclerenchyma zone; (3) cell division in the gall.

The term "nutritive zone" was used generally by most of the cecidologists before Beyerinck to indicate that this tissue nourished the larvæ and by most of the students after Beyerinck to indicate that this tissue was rich in nutritive substances. Just why does this tissue contain so much of these nutritive substances; *i.e.*, mainly starch (Figs. 5, 12, 13) and proteins (Figs. 8, 10)? When foreign substances attack plant tissues, there is a demand for an abundant supply at the place of irritation. The sap carries, besides many other substances, carbohydrates, aminoacids, polypeptides, and includes some normal unspecific protective substances. The latter tend to inactivate and convert the irritable substances into substances tolerable for the plant organism until the tissue acquires the potency to produce more, and to a certain extent specific, antibodies against the irritating substances. Parallel with this series of reactions in the plant tissue, many others develop. The carbohydrates undergo dehydration and form larger molecules. The tissue becomes rich in starch grains which grow larger and larger during the period of the irritation (compare Fig. 13*B*

with Fig. 12). Carbohydrates, aminoacids, and polypeptides build protein molecules which form protein grains. Some of the protein components are apparently used at the same time for the formation of protective substances. These series of reactions appear to be general phenomena always occurring when foreign substances are introduced into the living tissues. In this connection we want to recall the following points: the accumulation of the starch above the callus in interspecific and intergeneric grafting (Kostoff, 1928); the accumulation of starch in the integument around the nucellus when the nucellus envelops endosperm and embryo produced after species crosses (Kostoff, 1929); the accumulation of glycogen in human tumors (Sokoloff, 1926); and finally the accumulation of starch and proteins in the gall tissue around the center of irritation.

The enlargement of the cells of the nutritive zone is due to the abundant storage of nutritive substances. The agents primarily responsible for this enlargement are the foreign substances. These are also responsible for a decreased division of the cells in the nutritive zone, and for the gradual destruction and dissolution of the cell elements surrounding the center of irritation. The foreign substances responsible for these destructive processes are of enzymatic nature. The morphological change in the nutritive zone indicates that diastase is present among the various substances which enter into the plant tissue. The presence of diastase was proved by experiments described above. This enzyme hydrolyzes the starch gradually and continuously in a centrifugal direction from the larval cavity. In very young galls (Fig. 13*B*), its affect is not strikingly manifested. Complete hydrolyzation of the accumulated starch has occurred, however, in the cells closely surrounding the larval cavity in the more advanced stages of the gall (Fig. 12). In old galls (Fig. 13*A*), the starch is present only in the very outermost layers of the nutritive zone and in the sclerenchyma; the entire storge of starch is usually hydrolyzed at the time the insect is ready to leave the gall. Other destructive processes run parallel with that of starch hydrolyzation. The destruction of protein grains and nuclear membranes (Fig. 10) is followed by the destruction of the entire cell content. This destruction is a manifestation of the presence of the proteolytic

enzymes. A gradual and continuous hydrolyzation of the cell walls (cellulose and lignin) following this proteolysis indicates the presence of cytase and hadromase.

The presence of enzymes opens the question whether all the enzymes originate exclusively from the larvæ (larval secretion) or have other sources for their production. The presence of unicellular organisms in the larval cavity has already been mentioned. Brown (in Lutz and Brown, 1928) isolated a new species of bacteria, *Erwinia espinosa,* from the spiny aphid gall on witch-hazel (*Hamamelistes spinosus* S. on *Hamamelis virginiana* L.) and found it able to hydrolyze starch. Similar conditions to those in the galls are found in the bacterial nodules of leguminous plants where the lysins dissolving the nuclear membrane in the nodules (Fig. 15) are beyond doubt bacterial products. Since unicellular organisms are found in the plant tissues about the larvæ and in the larval cavity, one questions whether the agents responsible for the dissolution of the nuclear membrane in the cells of the innermost layers of the nutritive zone (Fig. 8) originate from the larvæ or from the unicellular organisms, or whether they arise from both sources. A definite answer to this question must await an exact experimental analysis; though from a comparison of the phenomenon which occurs in the nodules with the observation on the nutritive tissue of the gall, one may assume that the unicellular organisms in the galls play an important rôle during the dissolution of the cells of the nutritive zone. On the other hand, there is some evidence that the larvæ also excrete enzymes. Cosens (1912) has shown the presence of an enzyme, diastase, in a series of experiments with larvæ of *Amphibolips confluens.* A morphological condition, an eccentric utilization of the nutritive zone, often occurring below the ventral region of the larvæ would seem to give some morphological evidence for this excretion of enzymes by the larvæ.

The sclerenchyma zone occurs in most of the mature Cynipid galls and immediately adjoins the nutritive zone. This zone has been, and is still, very often called " the protective zone " by cecidologists. It is pointed out by some of them that it serves for the protection of the larvæ, by others that it serves for the protection of the plants. Some cecidologists now use the term

"sclerenchyma" for this zone, since its cells with their heavily sclerified (lignified) walls resemble most closely the stone cell type of sclerenchyma. These walls were found to stain a red-violet with phloroglucine-hydrochloric acid, a test for lignin. It appears preferable to use the term sclerenchyma. We cannot accept the teleological conception whereby this zone serves for protection either for the insect or the plant, but consider it to be a product of plant reaction where the interraction between foreign substances and the plant protective substances takes place.

In discussing the reason for the cessation of growth in the gall, we concluded that the cause was to be found at first in the appearance of a balance between the foreign substances coming from the larval cavity and the plant protective substances and later in an excess of the latter. Sclerification in young galls usually affects first a larger spheroidal area around the nutritive zone which is more than 4–5 layers in *Andricus* galls where the thick cell walls show lignification. This coincides approximately with the time at which intensive cell division ceases, *i.e.*, when inactivation is effected for all foreign substances outside of the nutritive zone. In a later stage, the sclerification is limited in *Andricus* galls to 2–4 layers immediately adjoining the swollen cells of the nutritive zone. This is the time when the plant has acquired the potency for higher production of protective substances which neutralize in a very small area the foreign substances coming from the nutritive zone.

Just how sclerification occurs is a question which cannot be answered with our present knowledge, but it is a matter of fact that it is morphologically manifested as a reaction product in the plant tissue where the plant substances inactivate the foreign substances into ones tolerable for the plant tissue. This appears to be a general phenomenon. The senior author has found that the nucellus which envelops endosperm and embryo obtained after species cross pollination in *Nicotiana* suffers greater sclerification than the nucellus which envelops endosperm and embryo obtained after self fertilization in a pure species. In the first case, the nucellus cells sometimes divide and form more than one layer of very heavily sclerified nucellus. The sclerification is much greater and affects a broader area in the region where the most

active exchange of substances between the maternal tissue and the hybrid endosperm and embryo occurs, *i.e.*, in the region of the chalaza and the region directly opposite it. Secondary thickening in the parenchyma also occurs in interspecific and intergeneric graft unions in a small area around the callus (Kostoff, 1928) as a mutual reaction between the scion and stock. Further, in the parenchymatic region in plant tumors, he has found that it occurs as a result of the plant reaction against the agents which cause the tumor. The xylem in the higher plants is subjected to this process of secondary thickening and is the tissue which carries the unelaborated non-specific substances for the plant organism. One may correlate here the abundance of sclerenchyma cells occurring in the cortex of dicotyledonous plants, where they develop by sclerification of the thin walled parenchyma, with the fact that the bark represents the region exposed during the whole life of the plant to the attacks of bacteria, fungi, etc. The haustoria of *Cuscuta europæa* when they enter into the tissues of *Urtica dioica* become tracheid-like and lignified (Haberlandt, 1909). There is in this instance also a mutual interactivity between the haustoria of *Cuscuta* and the living tissues of its host. Striking sclerification of the cell walls in the tissues surrounding embryos is a very common phenomenon (all nuts, fruits of the Rosaceæ, etc.). Since it is well known that an absolutely mathematical homozygosis does not exist even after selfing in self pollinating plants, one may always treat the embryo more or less as a foreign implantation for the mother organism and the sclerenchyma around it as an inter-reaction product. Moreover, the embryo is always the locus in the plant where certain specific, entirely different metabolic processes develop with specific metabolic products that increase the inter-reaction between the maternal and embryonic tissues.

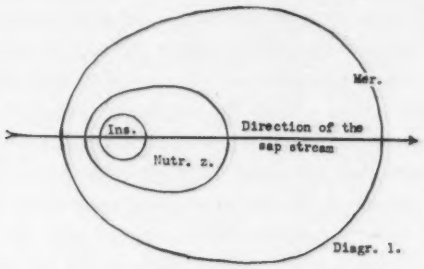
In closing the discussion of the appearance of sclerenchyma, one may formulate the facts in the following manner: everywhere foreign substances penetrate into the living tissue of higher plants, the plant reacts with formation of sclerenchyma. This can not be treated as a narrow dogma, since we do not think that everything is "inter-reaction," but, when one finds a correlative tendency in some phenomena, it is significant if one can show whether

such phenomena represent isolated occurrences or whether they are fundamentally similar.

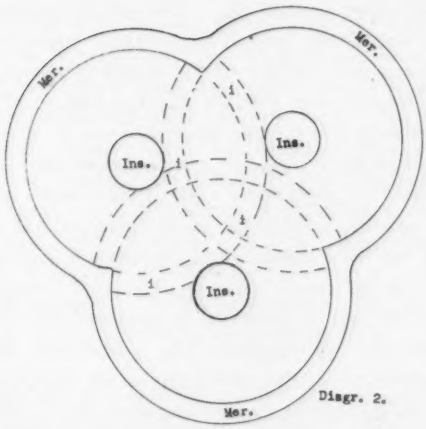
In very old galls, it was observed that a reduction in the number of layers of sclerenchyma occurs. In some cases, such as *Andricus palustris* where the separation occurs between the sclerenchyma and adjoining parenchyma, the reduction of the sclerenchyma layers can be treated sometimes as a purely mechanical compression caused apparently by the drying of the outermost layers so that the tangential walls of the cells adhere closely and appear in permanent preparations as a single very thick wall. This is not the only cause, for in other instances a reduction without compression was observed. Two factors may possibly be responsible for such a reduction of the sclerenchyma. One factor, the appearance of a balance of neutralization between the foreign substances and the protective substances produced by the plant and the subsequent localization of this process to a smaller area, has already been mentioned. The other factor may be the larvæ and unicellular organisms, both of which apparently produce the delignifying enzyme, hadromase. It is known that bacteria are capable of destroying and utilizing lignin (van Wisselingh 1925, etc.).

In the gall of *Andricus petiolicola* on *Quercus alba* where the petiole and twig are involved, there is an eccentric deposition of the zones about the larvæ (Fig. 1). The gall axis coincides nearly with the axis of the petiole or twig (diagram 1). The direction of the sap stream through the gall coincides with the gall axis, *viz.*, the sap comes first through the narrowest side of the zones. The deposition of the gall zones in such an ovoid form can be explained only on the basis of our general outline of the cause of gall formation. The irritable substances spread easier and to greater distances in the direction of the sap stream. Their inactivation occurs more quickly below the larvæ where the sap comes sooner, and more slowly in the opposite region above the larvæ, since the greater part of the protective substances is already used below the larvæ where the sap first meets the irritable substances. Thus, the protective substances are much more active below the insect where the sap stream passes first; this is later the place that less tissue is deposited. Foreign substances are much more active

above the insect and this is later the location of the broader region of tissues, where the sap stream passes later. In very early stages of gall development, the region with intensive cell division (the meristem) tends to form a spheroid at a certain distance from the



Diagr. 1.



Diagr. 2.

larva, since the foreign substances from the larval cavity tend to spread out radially at a uniform rate. If two or more larval cavities are located near each other, as often happens in the galls of *Andricus petiolicola* (Fig. 3), the meristematic regions between the larvæ come to overlap each other. These overlapping portions of the meristematic tissue between the two larvæ become the region of greater activity of the foreign substances which come from the adjacent larval cavities, and their cells lose the ability to divide (diagram 2, *i*); so that several adjacent larvæ often induce

such a composite region of meristem (diagram 2, *Mer*) as appears in Fig. 3.

In the meristem of some Cynipid galls, cell division is so intensive that the cells, though they continue to divide, do not succeed in reaching their normal size. In this manner, the meristem becomes composed of very small cells. The time soon comes when the plant succeeds in producing a large amount of protective substances and the inactivation of the irritable substances in the meristem takes place. With the increasing amount of the protective substances, the inactivation takes place in a small area just outside the nutritive zone and here the sclerenchyma begins to form. Cell division in the meristem ceases as soon as the elimination of the irritable substances takes place and the cells, now having sufficient food supply without stimuli for division, expand rapidly to reach their normal size. The result is a remarkable distention of all the tissues outside the sclerified region. This leads to separation processes outside of the sclerenchyma in certain leaf galls (*Andricus palustris,* Figs. 8, 12, 13), and a rupture of the tissues immediately outside the sclerenchyma in other leaf galls (*Amphibolips confluens,* Fig. 5; and *Andricus futilis,* Fig. 6).

The processes of separation and rupture were not observed in terminal bud and twig galls, since these have a greater sap supply which can bring considerable amounts of normal protective substances from the very beginning of the gall development and inactivate a great part of the irritable substances during the entire process of the gall development. As a result of this constant process of inactivation, the meristem of the terminal bud and twig galls is stimulated by smaller amounts of the irritable substances; there is less intensive division and fuller growth of the meristematic cells, due to this more active elimination of the irritable substances and to the more abundant supply of sap and nutritive substances, than in the case of the leaf galls. Here too, the plant finally acquires the potency to transform all the irritable substances outside the nutritive zone into tolerated and non-irritable ones. After this series of processes, which differ quantitatively from those in the leaf galls, the maturation process of the meristematic cells in the terminal bud and twig gall is not sufficient to effect separation or rupture since this process is gradual and not abrupt as in the leaf galls.

The appearance of irregular cell division such as retarded separation of divided chromosomes, binuclear and multinuclear cells, nuclear hypertrophy, etc., is common in the gall tissue under the influence of the foreign substances that penetrate into the plant tissues. These seem to be general phenomena wherever foreign substances attack living tissue. The senior author has found similar abnormalities in tobacco plant tumors. Smith, Brown, and McCulloch (1912) found giant cells with several nuclei, rapidly proliferating anaplastic cells, "amitotic division," and occasional abnormal mitotic divisions in crown gall tissues. They deduce then the "morphological likeness of crown gall to malignant animal tumors." In *Heterodera* galls on plants, multinuclear cells were found by: Němec (1904, 1910), Molliard (1900), Houard 1906), Tischler (1901), Küster (1916), etc. Multinuclear cells and other cellular abnormalities were found by Němec (1924) in galls caused by the Eriophyidæ. Kostoff and Kendall (1929) have found that when these gall mites attack flower buds of *Lycium halimifolium* irregular meioses occur. Abnormalities in cell division also appear when the tissue is exposed to definite chemical agents (Klebs 1896; Demoor 1895; Andrews 1905), to narcosis (Němec, 1910; Sakamura, 1920; etc.), to low temperature (Sakamura, 1920; Belling, 1925; etc.), and other conditions and factors. Plant and animal tissues react in the same way also when they are exposed to Radium and X-rays; the literature of the latter subject has been recently reviewed by Miss Paula Hertwig (1927).

An example of retardation of the chromosome separation in the gall of *Andricus petiolicola* is given in Fig. 23. The chromosomes are usually more highly inactivated on the side from which the foreign substances attack the cells. The chromosomes of the cells which are under the influence of these foreign substances are spherical or ovoid in permanent preparations just as they usually appear in the pollen mother cells and the embryo sac mother cells. A similar parallelism was observed by Cosens (1912) and Cosens and Sinclair (1916) concerning the appearance of trichomes on the gall and on the reproductive axes of *Acer*, etc.; the abnormal forms are usually found duplicated on the reproductive axes of the host.

Irregularities in cell division occur not only in the plant tissues but also in the animal tissues when they are under the effective influence of foreign substances. All that has been said about plant tissues is equally true for those of the animal. In Fig. 27, certain cell divisions with lagging chromosomes on the spindle are from cells of the larvæ of *Andricus palustris*. In some of the cells retardation of the chromosome separation is so great that tetraploid and octoploid nuclei are formed. Apparently, chromosome division without cell division goes so far in the cells about the mesenteron that giant cells about 200 times larger than the smallest cells of the larval tissue are formed (Figs. 14, 18). Similar somatic poliploidy was observed by Miss Holt (1917) in the alimentary tract of *Culex pipiens* where the number of chromosomes in the cells of the pupal intestine is considerably increased during metamorphosis. In *Culex*, the agents responsible for the foreign substances produced during the pupal stage are apparently bacteria which are active in the alimentary tract during this stage. However, during metamorphosis, there is always present in the pupal organism an abundance of lytic agents, and irritable, toxic, and otherwise intolerable substances. These latter represent the autolytic products from the larval cells. They are as capable as quite foreign substances from external sources in causing irregularities in the mitoses of certain cells. Toumanoff (1927) found that the honey bee was able to acquire immunity to *Bacillus alvei*, and cites the work of Metalnicoff and his students, where other insects were found to possess the ability to produce antibodies.

From the immunological standpoint, an organism produces antibodies against certain substances when they are parenterally introduced into the organism. The conditions, however, in which the Cynipid larvæ live are different. The larva is surrounded with all the products of dissolution from the plant tissue and the products of the unicellular organisms. Some of these products are very highly toxic. When some of them, substances with relatively small molecules, penetrate into the larval or pupal body, the latter does not always succeed in altering them into tolerable substances and they may possibly affect the cell division. This factor must be taken into consideration, as well as the more prominent activity of the lytic agents during the process of his-

tolysis in metamorphosis, as a cause of the irregularities of the cell division in the insect tissues.

There is, however, nothing to be gained for the explanation of gall formation in the suggestion of Magnus (1914): ". . . einer Gallwirkung durch Antikörper, welcher unter der pflanzlichen Stoffe selbst im Parasiten erzeugt werden . . ." (p. 142). He made this suggestion chiefly on the basis of the Beyerinck-Sachs hypothesis for gall formation: "Eine wesentliche Stütze für die Beyerinck-Sachsche Hypothese, dass wirklich die Gallen unter der Einwirkung bestimmter formbildender Stoffe entstehen, müsste aber der sichere Nachweis sein, dass auch in der normalen Entwicklung solche organbildenden Stoffe eine Rolle spielen" (Magnus, 1914, p. 142). To show that one can arrive at absurd conclusions when building upon such insecure foundations, we quote Wells' (1916) sentence: ". . . the germ plasm of the cecidozoön is the place of origin of the gall forms." This is in direct contradiction to the findings of immunology and genetics. Formative substances usually build characters and organs in the specific medium of the species. In species crosses, their formative potency is gradually diminished with the gradual decrease in the closeness of relationship between the parents. The crossing of very distantly related species and very nearly related genera may often produce hybrid embryos, but these die in very early stages as the result of formative and immunological causes according to unpublished observations of the senior author. This accounts for the rarity of genus hybrids.

On the basis of his statement just quoted, Wells (1921) derived the "Evolution of Zoöcecidia": Zoöcecidial evolution then is a complex in which, in its early stages (kataplasmas) with regard to certain characters, the plant's *germ plasm*[1] dominates, while in its later stages (prosoplasmas) the animal's *germ plasm*[1] gains control; the whole, however, constituting a single progressive series of factorial transformation as far as the changes in the animal *germ plasm*[1] are concerned" (p. 374, 375). It is difficult to conceive how any kind of germ plasmic control of the animal tissue over the plant tissue, or *vice versa*, can exist when as soon as foreign substances are introduced into an organism the latter strives to change the former as soon as possible.

[1] Italics are the authors.

In conclusion, on the basis of the present studies and those of various previous authors, one can formulate the general morphological appearance of the Cynipid galls as a reaction product of the plant tissue dependent on:

1. Plant specificity, including the amount of normal protective substances in the plant, and the plant potency of acquiring protective substances against invading foreign substances.

2. The quantity of sap entering the organ or tissue where the gall originates.

3. The specificity of the insect, which one considers in the quality of the foreign substances (excretions) originating from this source.

4. Quantity of the foreign substances.

5. Mechanical injuries and their disintegration products.

6. The presence of unicellular organisms and the activity of their products, qualitative and quantitative.

Cynipid galls appear as a reaction product of all these factors and not as a result of any one factor, nor does it seem plausible to consider evolutionary concepts such as those advanced by Wells (1921) in their formation.

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DESCRIPTIONS OF PLATES.

Abbreviations.

L—larva.
M—meristematic region.
N—nutritive zone.
P—parenchyma zone.
V—vascular
S—sclerenchyma zone.
a—cells of mesenteron.

PLATE I.

FIG. 1. *Andricus petiolicola* on *Quercus alba*. A section of larval chamber showing eccentricity of the tissues about the larva, the greatest amount of nutritive as well as other tissues being away from the central axis of the gall, and meristematic region (M).

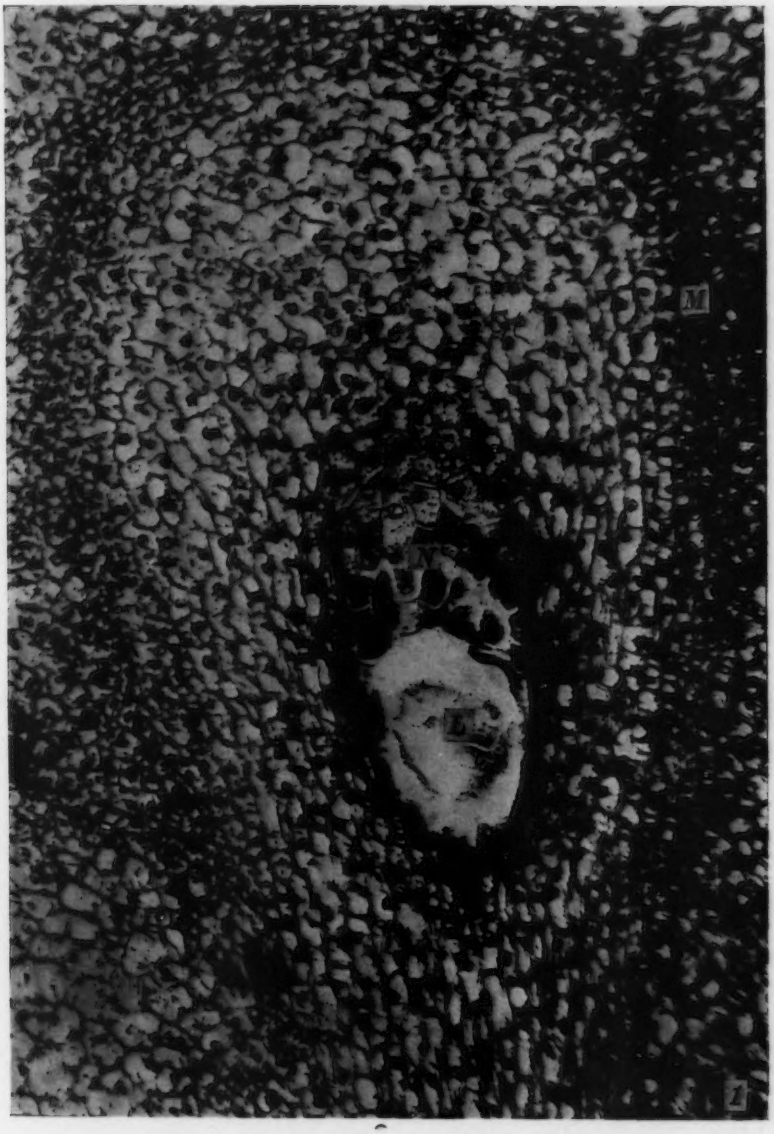

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PLATE II.

FIG. 2. *Neuroterus batatus* form *bisexualis* on *Quercus alba.*

FIG. 3. *Andricus petiolicola* on *Quercus alba.* A section of a younger gall than the one in Fig. 1 showing the meristematic regions about the chambers fusing and appearing as a curving band of very deeply stained cells (M).

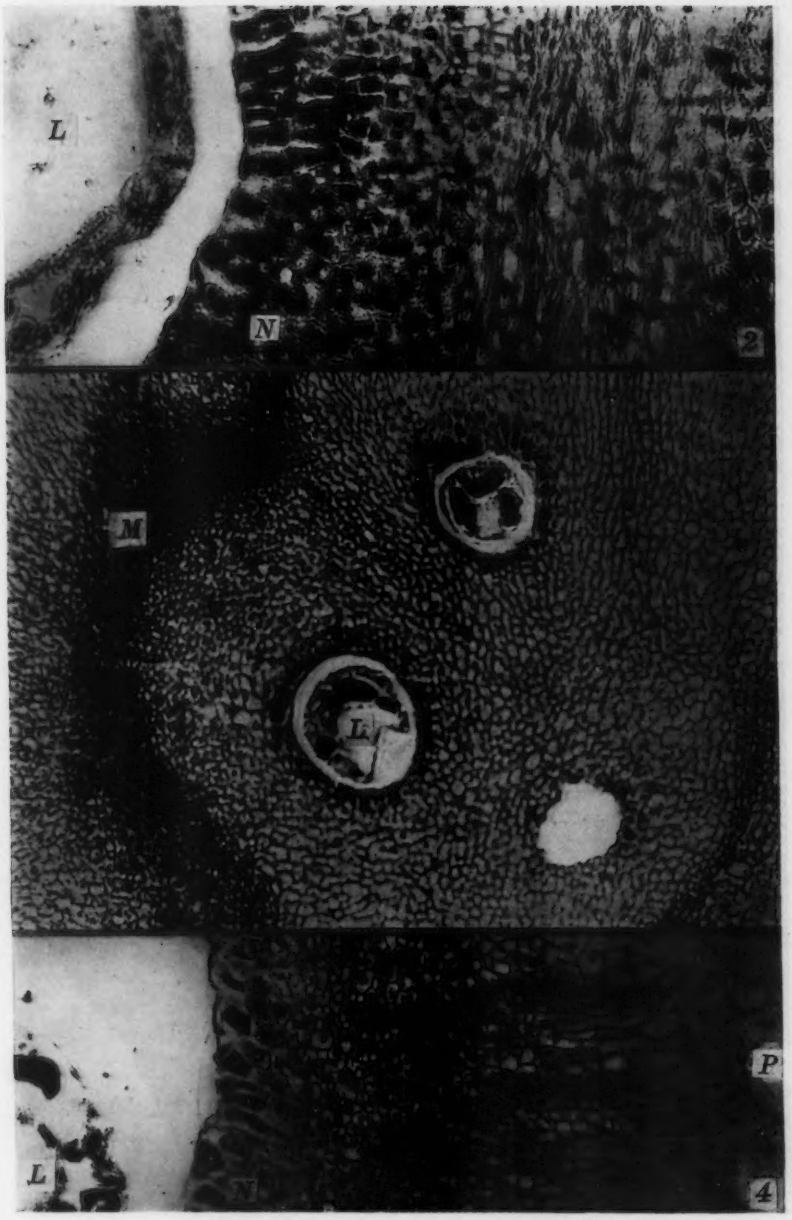

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PLATE III.

FIG. 4. *Amphibolips confluens* on *Quercus velutina*. A section of a very young gall (about 5 mm. in diameter).

FIG. 5. *Amphibolips confluens* on *Quercus velutina*. An iodine preparation of a section of a gall about 12 mm. in diameter showing the starch accumulation in the cells of the outer layers of the nutritive zone and in the cuboidal cells, between it and the parenchymous strands, which become increasingly lignified and come to form the sclerenchyma zone.

FIG. 6. *Andricus futilis* form *futilis* on *Quercus alba*. A section showing a portion of one larval chamber of sclerenchyma and nutritive zones suspended by strands of the torn parenchyma tissue.

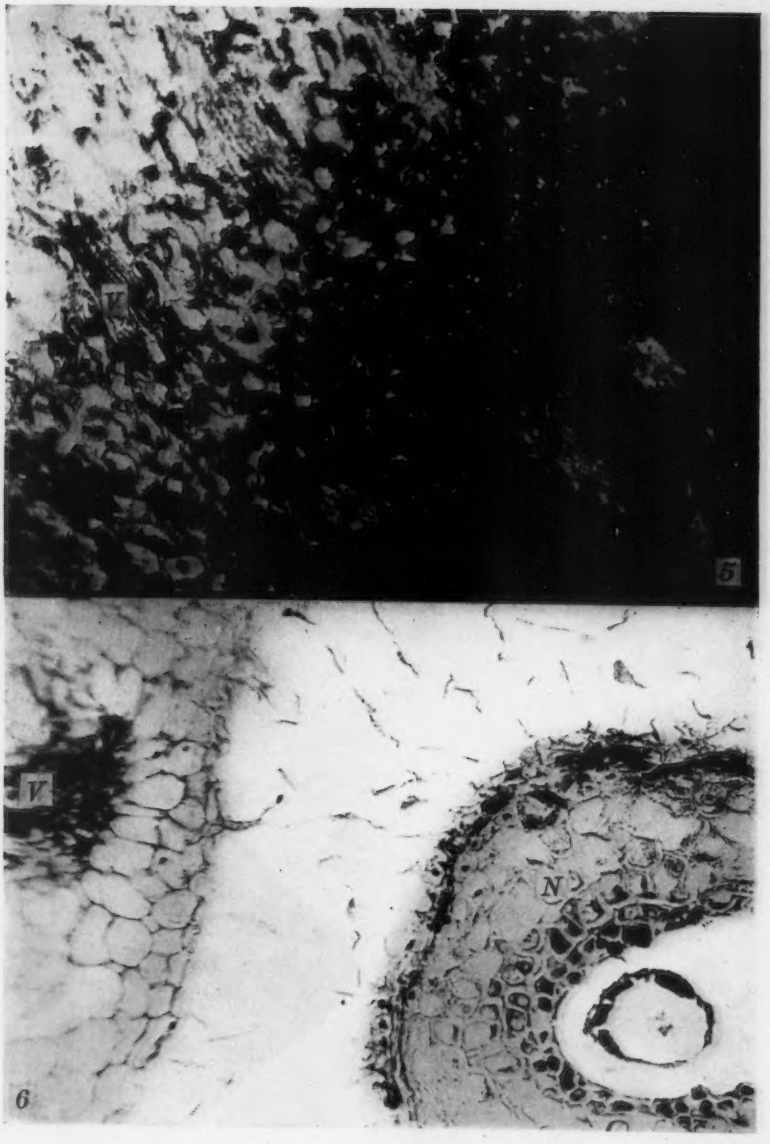

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PLATE IV.

FIG. 7. *Andricus petiolicola* on *Quercus alba*. Some cells from the nutritive zone of section shown in Fig. 1 showing bacteria-like content.

FIG. 8. *Andricus palustris* form *palustris* on *Quercus palustris*. A section showing the beginning of the separation between the sclerenchyma and parenchyma zones in the region of small undifferentiated cells.

FIG. 9. *Neuroterus minutus* form *minutus* on *Quercus alba*. A section showing the cells of the nutritive zone enclosed in a narrow zone of sclerenchyma with an outer zone of elongated parenchyma cells.

FIG. 10. *Andricus palustris* form *palustris* on *Quercus palustris*. Several cells from the innermost layers of the nutritive zone showing the fragmenting nuclei.

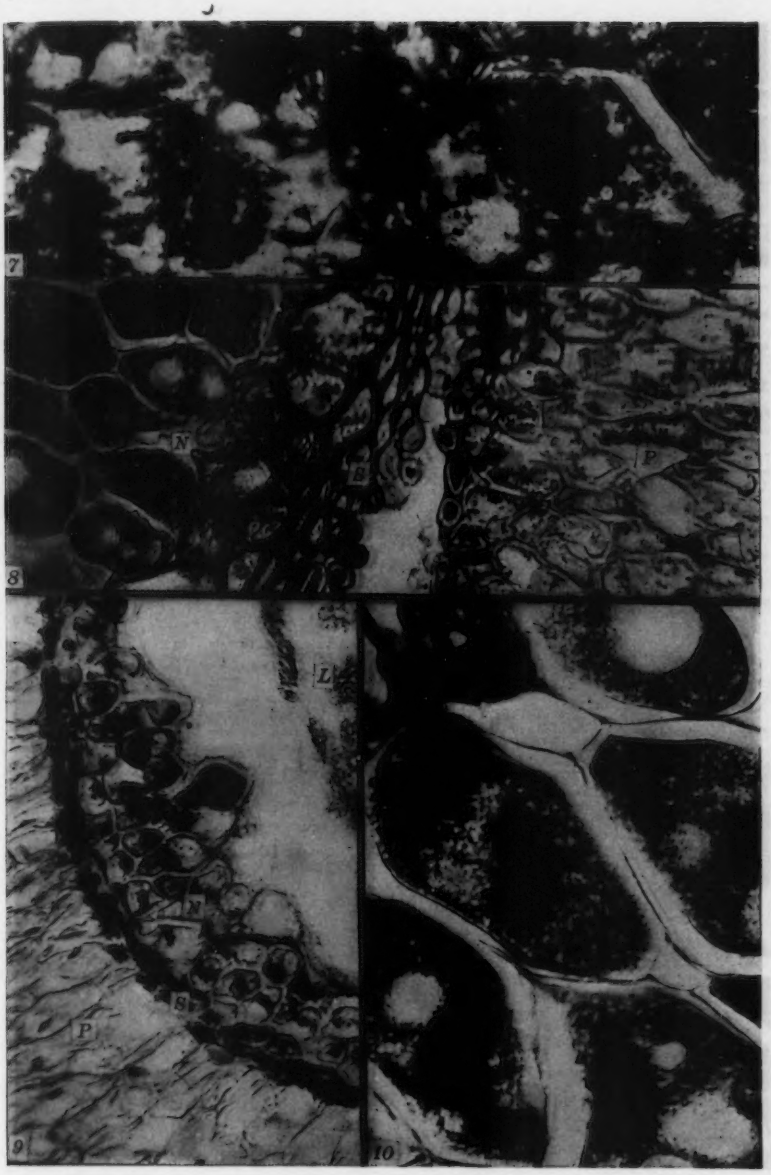

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PLATE V.

FIG. 11. *Neuroterus minutus* form *minutus* on *Quercus alba.* Portions of three larval chambers showing various degrees of utilization of the nutritive zone; the upper one showing an unequal progress of dissolution.

FIG. 12. *Andricus palustris* form *palustris* on *Quercus velutina.* An iodine preparation of a section of the gall showing the distribution of the starch at the time of separation of the two inner zones, which contain starch, from the two outer ones of parenchyma and epidermis, which do not contain any starch.

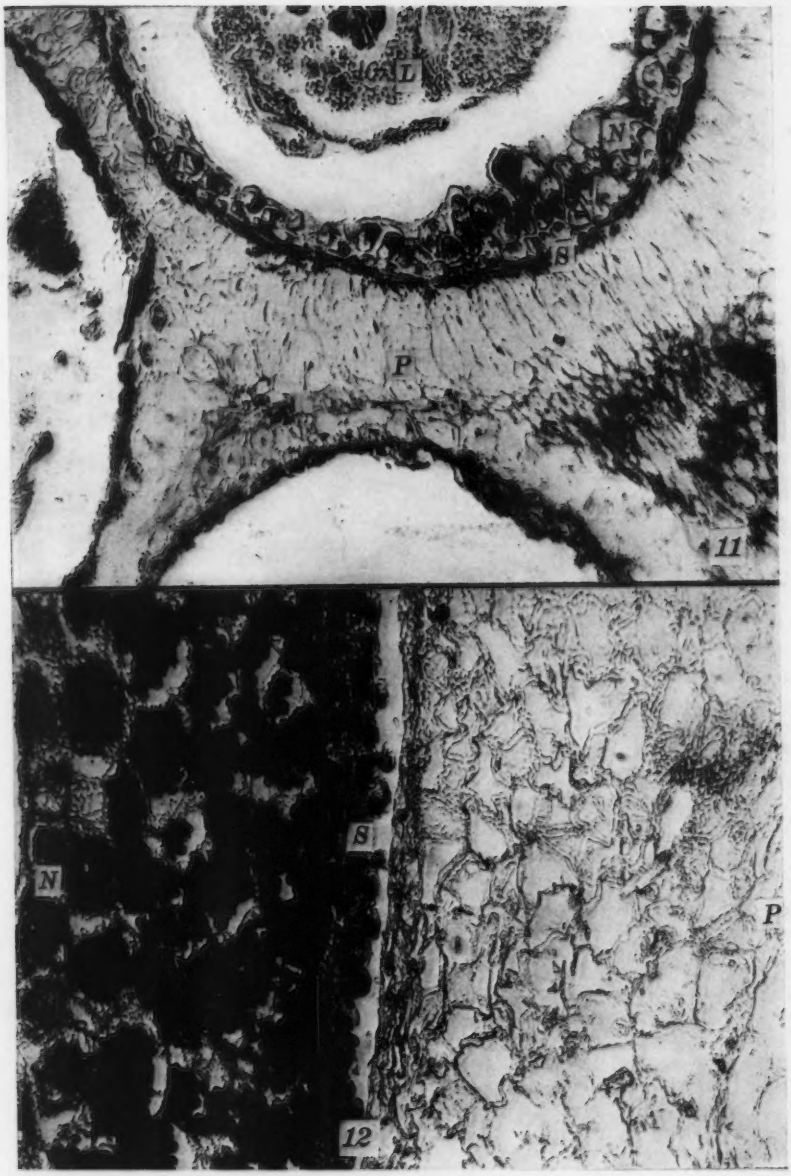

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PLATE VI.

FIG. 13*A*. *Andricus palustris* form *palustris* on *Quercus ellipsoidales*. An iodine preparation of a section of the larval chambers of two galls of different ages; the younger gall represented on the left.

FIG. 13*B*. *Andricus palustris* form *palustris* on *Quercus Ludoviciana microcarpa*. An iodine preparation of a section of a gall before the separation of the zones showing the distribution of starch.

FIG. 14. A section of the larva of *Andricus palustris* form *palustris* showing two very large cells (*a*) of the mesenteron wall, and the comparatively small cells of the adjoining tissues.

FIG. 15. Cells from the nodules of *Phaseolus vulgaris* caused by nodule bacteria showing different degrees of dissolution (destruction) of the nuclear membrane.

Remark: Figs. 13*A* and 13*B* are less magnified than Fig. 12.

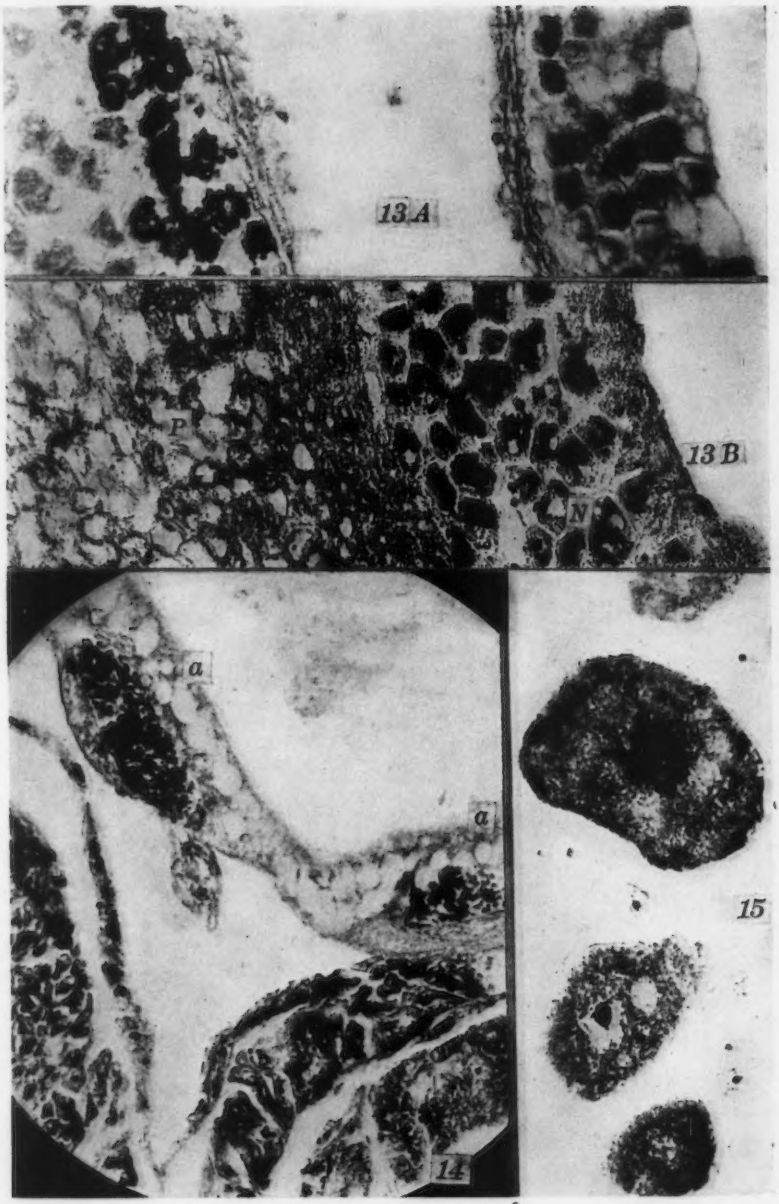

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PLATE VII.

FIG. 16. Grains of potato starch hydrolyzed by treatment with the extraction of the nutritive zone of gall of *Amphibolips confluens* on *Quercus velutina*.

FIG. 17. *Andricus palustris* form *palustris* on *Quercus Shumardii*. Cells of the innermost part of the nutritive zone showing the increasing size of the nucleolus as the larval cavity is approached.

FIG. 18. Cell from mesenteron wall (*a*) in an older larva than shown in Fig. 14, with adjoining tissue cells, adipose, etc.

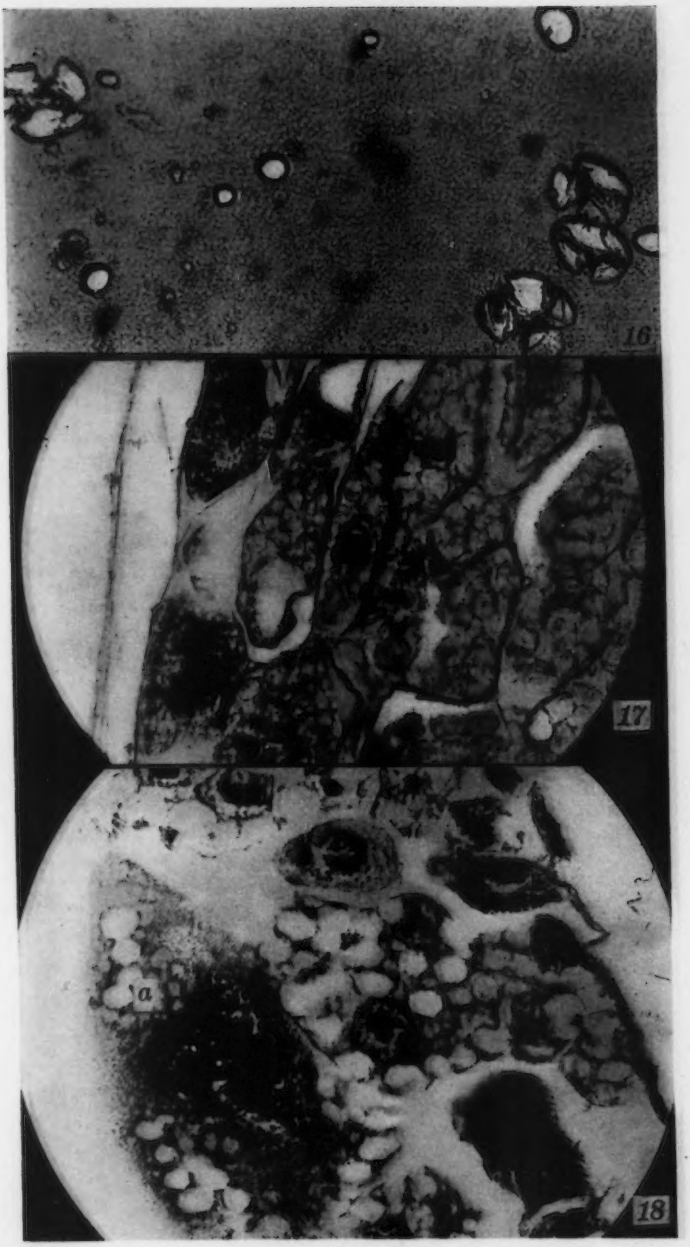

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Plate VIII.

Fig. 19. Sclerenchyma zone (S) of the gall of *Andricus palustris* form *palustris* with adjoining layer of nutritive zone (N) showing the relative abundance of binucleated cells of the former. Camera lucida drawing with 4 mm. obj. and 12.5 × occ.

Fig. 20. Metaphase from the cells of the larval tissues of *Andricus palustris* form *palustris* with 10 chromosomes. Camera lucida drawing, 1.9 mm. obj. and 12.5 × occ.

Fig. 21. Incomplete nuclear division in cell from the region just inside the region of most intensive cell division in gall of *Andricus petiolicola* on *Quercus alba*. Camera lucida drawing, 1.9 mm. obj. and 12.5 × occ.

Fig. 22. Two binucleate cells from the inner region of radiating parenchyma in gall of *Amphibolips confluens* on *Quercus velutina*. Camera lucida, 1.9 mm. obj., 12.5 occ.

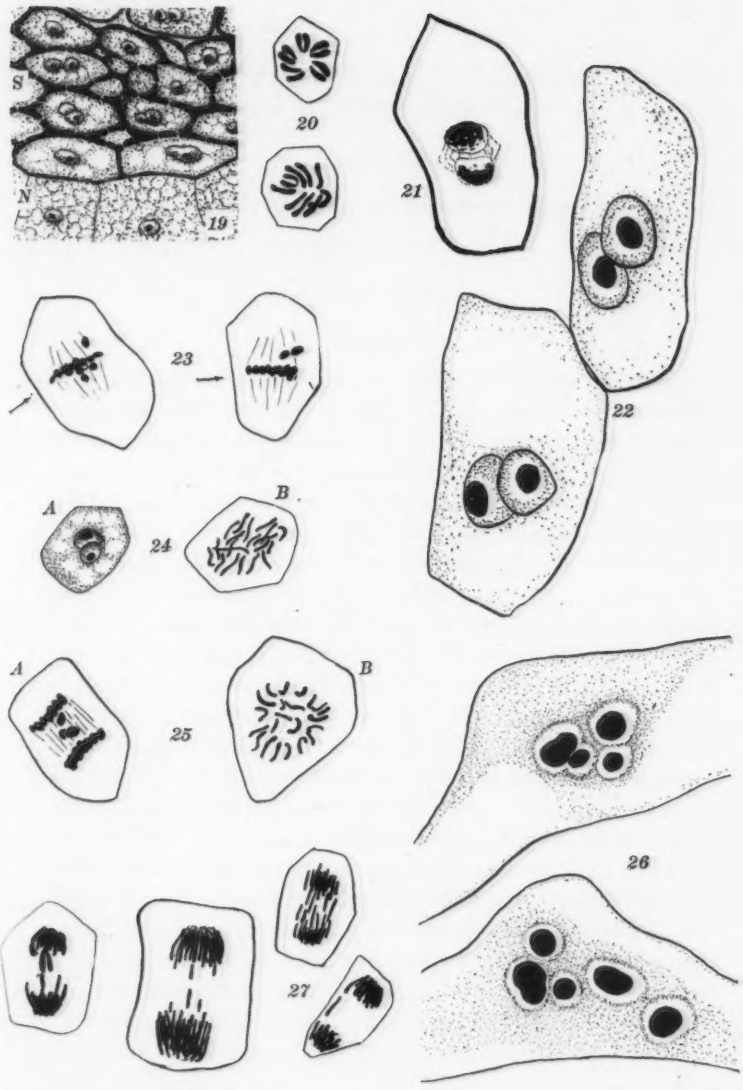
Fig. 23. Cells from same gall as Fig. 21, but from region of intensive cell division; arrows indicate direction of stimulus centrifugally from larval chamber with complete inactivation of chromosomes toward it and lagging of those away from it. (Camera lucida, 1.9 mm. obj., 12.5 occ.)

Fig. 24. Cells from the larva of *Andricus palustris* form *palustris*. *A*—Binuclear cells from the larva. *B*—Cell from the same larva with 20 chromosomes. Camera lucida, 1.9 mm. obj., 12.5 × occ.

Fig. 25. Drawing from the cells of the gall of *Neuroterus batatus* form *bisexualis* on *Quercus alba*. *A*—Cell from region of intensive cell division showing the chromosomes lagging on the spindle; chromosomes ovoid or round. *B*—Metaphase of cell from region of the plant tissue beyond the influence of the insect larva; the chromosomes are prolongated and slender as they usually appear in normal somatic cells. Camera lucida, 1.9 mm. obj., 12.5 × occ.

Fig. 26. Two polynuclear cells from the tissue about the canal of the gall of *Amphibolips confluens* on *Quercus velutina*. Camera lucida, 1.9 mm. obj., 12.5 × occ.

Fig. 27. Irregular cell division in the larvæ of *Andricus palustris* form *palustris* from various regions of the body. Camera lucida, 1.9 mm. obj., 12.5 × occ.



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THE EFFECTS OF BILATERAL OVARIOTOMY IN THE BROWN LEGHORN FOWL.[1]

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TWO PLATES (4 FIGURES).

INTRODUCTION.

In a previous report (Domm, '27*a*) the effects of complete ovariotomy were recorded in detail. It was shown that following the total ablation of the normal left ovary in the brown Leghorn fowl the right gonad, which normally is a minute rudiment, hypertrophies and forms an organ usually of testis-like form and structure. In these earlier cases, where the operation was performed at a relatively late age, these testis-like right gonads were always sterile. In a subsequent series (Domm, '28, '29), in which the operations were performed at a very early age, some of the hypertrophied testis-like right gonads thus far examined reveal active spermatogenesis, though in other respects, relating principally to secondary sexual characters, these individuals are essentially similar to those described in our earlier series (Domm, '27*a*). The rudimentary wolffian ducts respond to the presence of these testis-like gonads by growth and often by coiling. The left oviduct, in the absence of the ovary, shows varying degrees of reduction. Such birds develop the secondary sexual characters of the male. Hence the plain female plumage is gradually replaced by the more gaudy male plumage (see bird no. 1018, Plate 1) but this is subsequently replaced by henny plumage when the testis-like gonads attain sufficient activity (see bird no. 845, Plate 1). The head furnishings usually relatively small and fine in texture after a time, varying somewhat in different individuals, become large,

[1] This investigation was supported in part by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

coarse, and male-like (see Plate 1). Spurs develop and attain varying dimensions (see Plate 1). Behavior is likewise modified. The normal female is comparatively peaceable. Following the operation many of them eventually become pugnacious, acquire an interest in the female, crow, and attempt to tread.

The experiments on castration and those on transplantation of male gonads in the cock (Domm, '27*c*, and others) have shown that the testes have a pronounced effect on the development of the dependent sex characters. Hence in the presence of the male gonad the dependent characters, including head furnishings, behavior, and to a lesser degree the sexual ducts, are well developed while in the absence of the male gonad they become reduced and inconspicuous or disappear (for type of head furnishings in capon see bird no. 608, Plate 2). The development of certain male characters in the poulard, such as masculine head furnishings, behavior, and growth of the wolffian ducts was therefore attributed to the presence of the testis-like compensatory gonad found on the right, or a similar gonad occasionally regenerated on the site of the removed left ovary (Domm, '27*a*). In order to verify this supposition it was essential to remove these gonads and observe the effects on these characters.

Benoit ('23) performed an operation of this type on a white Leghorn female when approximately 6 months old. This bird had been previously ovariotomized at the age of 26 days. Whether this operation was completely successful in removing all gonad tissue beyond the possibility of subsequent regeneration is not known, as the bird was not kept a sufficient length of time, though from our experience we would surmise the contrary. Zawadowsky ('26) likewise reports a case in which he endeavored to remove the right testis-like gonad. The ovary had been previously removed on November 15, 1919 (age not given), and on May 18, 1922, approximately 2½ years later, the bird was opened on the right and the oval testis-like gonad incompletely removed.

In our earlier series of ovariotomy experiments (Domm, '27*a*) attempts were made to remove some of the hypertrophied right gonads by secondary operations. However, removal of the gonad at this late stage in its development was found to be difficult and exceedingly hazardous owing to its firm consistency and its posi-

tion over, and close adherence to the large post caval and right iliac veins. These gonads are never attached by a narrow mesorchium, as is the normal testis, but they invariably show a more diffuse area of attachment rendering their extirpation extremely hazardous. A considerable number of extirpations of the fully formed gonad were attempted. However only a few of these attempts were completely successful in the sense that no regeneration of the gonad had subsequently occurred as witnessed by post-mortem examination.

The hazards involved in completely disposing of all gonad tissue once the organ has fully hypertrophied, allied with its subsequent regeneration and growth, made its advisable to perform this operation at a much earlier age before the right rudimentary gonad had shown any appreciable hypertrophy. In the normal female fowl the rudimentary right gonad consists merely of a long, narrow, flattened sheet of tissue extending posteriorly from the median border of the right adrenal on the vena cava and junction of the right iliac veins. Following ovariotomy its growth is usually negligible macroscopically prior to the third week. Attempts to remove it surgically in this state would essentially be equivalent to an attempt to remove a part of the wall of the post caval itself. A new technique was therefore devised by which the rudimentary gonad was destroyed prior to hypertrophy by means of a small electric cautery. This method differs from that previously employed only in the destruction of the gonad at an early stage by cautery rather than its much more hazardous surgical removal at a later stage. This method has several obvious advantages. It enables one to perform the operation anytime prior to, or shortly following, ovariotomy. The operation also has the pronounced advantage of being much less hazardous for with moderate care one may completely destroy the rudimentary gonad with no, or very little, hemorrhage, a state of affairs practically impossible with the former method. Furthermore, any small part of the gonad not destroyed by the initial operation may readily be destroyed by a subsequent cauterization.

This investigation is part of a larger program on the biology of sex now being pursued at this laboratory under the direction of Prof. Frank R. Lillie. Acknowledgments are due Prof. Lillie for his assistance in making this study possible.

Experiments.

Thirty birds of the same age and approximately the same size were selected for this experiment. These were ovariotomized late in the summer and early fall of 1926 at ages ranging from 76–79 days. Each of these birds was subsequently opened on the right side between 16 and 22 days following the initial operation (see table) and the rudimentary gonad destroyed by thoroughly searing with a small electric cautery. Nineteen of the birds thus operated form the basis for this report while the findings in the rest of the series will be recorded at a later time. No difficulty was encountered during the course of this new method of operation. With very few exceptions the operation was completed with no, or very minor, hemorrhage and in most instances the bird was in good condition and recovered rapidly following the operation. In a few cases where recovery was slow the difficulty could be traced to the initial, more severe, sinistral operation.

Results.

In the experiments forming the basis for the present report the birds were deprived of both right and left gonads practically simultaneously; hence no dependent male characters ever developed. The changes observed following these operations coincide more closely, in some respects, with those *immediately* following sinistral ovariotomy than with those observed where the secondary dextral ovariotomy is performed at a much later time when the bird had assumed masculine characters. In the latter case there is usually a rapid decrease or disappearance of the dependent male sex characters, such as head furnishings and behavior, associated with a reversion to male plumage if the bird had assumed female plumage prior to the operation (Domm, '27*a*). A general categorical description of the effects of these operations will be given. All individual cases can obviously not be described in detail hence the description given in the text will be of a general nature with frequent reference to the tables to which the reader is referred for detailed case histories. For details on methods of operation, records, and preservation of materials the reader is referred to an earlier paper (Domm, '27*a*).

Effect on Head Furnishings.—The birds selected for these operations were relatively young growing pullets whose head furnishings at the time of operation were juvenile and had not yet attained very prominent proportions. The head furnishings therefore did not show a reduction in size as is frequently the case in the pullet ovariotomized at a later age when these characters have become relatively prominent. In a few instances where these characters were conspicuously red at the time of operation they became noticeably pale subsequently. In the completely successful cases these characters showed a slight gradual increase in size corresponding, in all probability, to the general increase in body size of the growing bird, until the bird matured, following which there is but little fluctuation in size (see table, cases no. 875, 876, 882, 884, 895, 897, etc.). Hence such a mature female completely deprived of all gonad tissue shows small, usually pale, comb, wattles, and earlobes (see bird no. 876, Plate 2).

In a number of cases the head furnishings later became red and turgid and manifested signs of growth though the size attained varied in each instance (see table, cases no. 874, 878, 880, 893, etc.). It is interesting to note that in each of these cases this growth was subsequently followed by a marked decrease. In a few cases this decrease was probably due to the general condition of the bird as it will be noted by reference to the table that a number of these birds died. In a few of the cases it may justly be attributed to this cause as the birds were sick for some time prior to the time of death (see table, cases no. 874 and 878). However one of the birds died because of crop-binding in which case it was afflicted for but a short period and when killed showed no symptoms of organic disease (see table, case no. 898). A few of these lived to the termination of the experiment and were killed in good condition (see table cases no. 880 and 893). Hence this reduction in size of head furnishings is not attributable in all cases to the poor physiological condition of the bird, though this is very frequently the case (see Domm, 27*a*), but must be imputable to other causes.

It will be noted by reference to the table that each of the cases showing conspicuous growth of head furnishings also reveals varying amounts of testis-like gonad. It is significant that such

birds may show the small capon-type head furnishings for practically 1½ years and subsequently show pronounced development of head furnishings indicating regenerating gonad (see table, case no. 894). This bird, whose complete history is given in the appended table, showed small capon-type head furnishings for 18 months following the operation, indicating a successful operation, which later became red, turgid, and conspicuously prominent. Bird no. 890 is exceptional and warrants mention in this particular. The head furnishings of this bird showed conspicuous growth between the 5th and 8th months following the operation (see table) after which there was a slight decrease. Post-mortem examination revealed no gonad tissue on either gonad site indicating successful removal. It is very probable that the growth observed here was due to an accidental autoplastic ovary graft which was being resorbed when the head furnishings began to decrease hence it could not be found at the autopsy.

Effect on Plumage.—The general differences in plumage exhibited by the sexes of the domestic fowl are fairly well known. For details of the plumage dimorphism, which is very pronounced in the light brown Leghorn, the reader is referred to an earlier paper by the writer (Domm, '27*a*).

The birds used in the present experiment had assumed the early female plumage at the time of operation. Following the operation they developed the juvenile plumage of the male to varying degrees (see table). These feathers are particularly conspicuous on the back, saddle, and wing areas. The new feathers on the breast and laterally, while juvenile for a time after the operation, early became black as in the mature male. This precocious development of definitive male feathers in these areas coincides with their earlier development in these regions in the male. However new juvenile feathers did not appear indefinitely in any region of the body for by 4 to 6 weeks after the operation the new ingrowing feathers were of the definitive male type in all areas. Mention should perhaps be made of the fact that the juvenile plumage is not lost as soon as adult plumage begins to appear but that this is usually a very gradual process, hence the presence of juvenile plumage at 6 months after the operation as the table reveals in a number of cases. Unfortunately the table does not give changes

between 6 and 12 months, if it did it would reveal the fact that some of these individuals retain this type of plumage even longer than 6 months. In exceptional cases where birds are in poor physiological condition and fail to mature before the onset of cold autumn weather these feathers may be retained until the following spring. By two to three months after the operation most of the birds in the experiment presented a conspicuous plumage consisting of some scattered old female feathers, a few scattered feathers showing female tips and male bases, particularly conspicuous on the breast and lateral areas, numerous juvenile feathers conspicuously confined to the back, saddle, and wing areas, and many adult male feathers most abundant on the breast and laterally but not by any means inconspicuous in all other areas. By five to six months after the operation the plumage had become completely male in all areas with some scattered juvenile feathers on the back, saddle, and wing areas in some cases (see table). If the operation is completely successful such a bird develops and retains (in these cases approximately 2½ years) a brilliant, luxuriant, adult male plumage (see table cases no. 875, 876, 882, 884, etc.), showing no subsequent reversion to female plumage as does the female in which merely the functional left ovary has been removed (see bird no. 845, Plate 1, also Domm, '27*a*). The successful bilaterally ovariotomized female therefore approximates the castrated male in this particular (compare birds nos. 876 and 608, Plate 2).

In several cases (see table, birds no. 880, 891, 893, 894) the bird developed male plumage for a time following the operation and later reverted to female plumage. Reference to the table will reveal that in each of these cases this reversion in plumage was preceded by considerable increase in the size of the head furnishings which in turn disclosed the presence of regenerating gonad. Bird no. 891 revealed such a reversion in plumage following considerable increase in size of head furnishings. Later these again decreased in size following which the new plumage again became male in character. About this time (October 16, 1927) a dextral laparotomy was performed, revealing a small mass of right gonad which was thoroughly seared. During the following 16 months to the time the bird was killed (March 1, 1929) the plumage remained male in character and the head furnishings small and

capon-like. Post-mortem examination revealed no gonad on either right or left sides. In bird no. 880 there was likewise a reversion in plumage to the female type following considerable growth of head furnishings followed by a reversion back to male after these characters had regressed for a time. Birds no. 893 and 894 showed a similar reversion to female plumage, though at widely different intervals, but differed from the above in that they did not revert back to male again but they probably would have done so given sufficient time correlated with no gonad activity as evinced by decrease in head furnishings.[1] Birds no. 874, 878, and 898 developed and retained male plumage to the time of death. In each post-mortem examination revealed some gonad (see table) correlated with growth of head furnishings which was most pronounced, though of short duration, in bird no. 898 and negligible in each of the others.

Effect on Spurs.—The effects of castration on the spurs of the male are incompletely understood. The Leghorn capon in our experiments always has well developed spurs (see bird no. 608, Plate 2). They however do not seem to grow any longer than those of the normal cock. The only appreciable difference we have been able to observe concerns their development. The spurs of the capon become sharp and pointed early in their development while those of the normal male remain stout and blunt for a considerable period, sometimes well into the second year.

The normal female of the Leghorn breed usually lacks spurs

[1] The gonadal plumage relationship in the poulard is evidently explicable on a quantitative basis. In the absence of gonad the female develops and maintains cocky plumage; the presence of small amounts of regenerating gonad does not alter this condition. As the mass of regenerating gonad gradually increases in size and presumably also in hormone production it eventually attains the point where it partially inhibits male plumage, and intermediate plumage develops. If the volume of hormone produced becomes still greater it ultimately attains the point where it completely inhibits male plumage and female plumage develops. This is to be explained by a secondary gradual development of female hormone as will be described in other studies by Domm and Gray. The parallel regression of head furnishings and secondary reversion of plumage from female to male in the cases described above indicate that the hormone production of the compensatory gonads was not much above the threshold of effectiveness and was readily depressed below it by sickness or lowering of vitality of the bird. The same principle was found to apply in our cases of sinistrally ovariotomized birds previously described (Domm, '27*a*).

though cases are known where otherwise normal females showed well developed spurs (cf. Domm, '27*a*, p. 86, also Goodale, '16). The young pullet of our breed shows no spurs, though one may always identify the small, oval, imbedded, spur rudiment on the inner surface of the shank. These rudiments gradually become more conspicuous throughout the life of the fowl though in no instance have we observed them more than a few millimeters in length in the old spurless hen.

Following sinistral ovariotomy in the Leghorn spurs have developed in all cases without exception (see birds no. 1018 and 845, Plate 1). The rate of growth and the ultimate size attained varies somewhat in individual cases though relatively well developed spurs are nevertheless the rule in all such birds. The development of spurs in the bilaterally ovariotomized birds of this series differed in no important respect from those in which merely the left ovary had been removed (see bird no. 876, Plate 2). Individual differences occur though the size attained in individuals of comparable age is apparently no greater in the one group than in the other. The table gives the length of the spurs in centimeters at the time of autopsy. For data on spur growth following sinistral ovariotomy the reader is referred to a previous publication (Domm, '27*a*).

It was pointed out above that the spurs of the capon become sharp and pointed earlier than those of the normal cock. Indications are that the spurs of the sexless female may not become pointed as early as those of the capon in at least certain individuals. Birds no 902 and 903 (see table) show spurs at 9½ and 8½ months following operation which are well rounded and blunt at the ends. On the contrary bird no. 878 (see table) shows sharp pointed spurs at 10 months. All individuals sacrificed toward the termination of the experiment had well pointed sharp spurs though the actual length attained varied considerably.

Effect on Voice and Behavior.—It is generally conceded that the normal female does not tread, that she is less combative than the male and that she does not crow. It is likewise held by many that the capon does not tread nor crow and that he is inclined to be non-combatitive. Following sinistral ovariotomy the female may develop the behavior of the male to a striking degree (see Domm.

'27*a*). Such a bird will crow, fight the male, call the female to an alleged morsel of food and attempt to tread but, while these reactions are very common and characteristic, instances where such a bird will actually tread are apparently very exceptional. It was previously pointed out (Domm, '27*a*) that when such an ovariotomized fowl is later completely castrated she loses her masculine voice and behavior and becomes capon-like.

In the present experiments the birds were bilaterally ovariotomized at a relatively early age. In cases where this experiment was completely successful, in disposing of all gonad tissue so that none regenerated subsequently, the bird never developed or exhibited the masculine voice or behavior. Such a bird develops much as does the capon in this respect. They were found to be relatively inactive and non-combatitive. They are noticeably more quiet than the normals of either sex. It was further observed that they will not receive or interest themselves in the male but are rather inclined to avoid him. They were never observed to brood, neither did they build nor sit on the nest nor did they appear to interest themselves in chicks; however the broody instinct has practically been lost in the variety of brown Leghorn with which we are here concerned. The broody instinct has been manifested in but a few cases in our flock of normal hens and communication with fanciers on this point indicates that this instinct is nearly lost in certain varieties of Leghorns at least. Hence this reaction is probably not to be expected in the sexless females in this experiment. Birds in which the operation has been incomplete as witnessed by the presence of regenerating gonad of testis type exhibited degrees of masculine behavior no different from that found in the sinistrally ovariotomized fowl.

Effect on size.—The size differences between the sexes of most breeds of fowl are well recognized. The Leghorn breed does not appear to be exceptional for here the male is conspicuously larger than the female. The stance in the two sexes also differs. The cock is characterized by a noticeable upright stance while that of the female is more horizontal. It is quite generally conceded that the male fowl when castrated, at a relatively early age, grows larger than the normal. The normal Leghorn capon in our flock has grown somewhat heavier than the normal cock but whether this is

due to excessive accumulation of fat, for which the capon is renounced probably due to his lethargy, or to an actual increase in skeletal proportions we have as yet not established. The probabilities however are that both factors are involved. Following castration the stance of the cock is altered, becoming horizontal so that it approximates more nearly that of the female.

No actual measurements have been made to show whether the fowl shows any changes in size of skeleton following sinistral ovariotomy. The general impression is that the poulard is no larger than the normal hen. Goodale ('16) contends that the poulard probably does not exceed very appreciably, if any, the size of the normal hen. He admits that his data have not been of sufficient extent, nor his stock sufficiently homogeneous in respect to weight, to give results of value. Finlay ('25) states that the ovariotomized female (sinistral) retains the skeletal characters and body shape of normal females but gives no measurements to substantiate his contention. We have found exceptional cases (Domm, *'27a*, also unpublished data) where the poulard was noticeably larger though in general we are inclined to believe that such size changes, while they may occur, are in most instances negligible. Furthermore since sinistral ovariotomy in the fowl does not produce a gonadless bird, as was formerly supposed, it is perhaps not to be expected that she would change appreciably in size if at all.

Size differences in the bilaterally ovariotomized Leghorn in our experiments deviating from the normal are not very conspicuous if they occur at all. In fact we are inclined to believe that they do not change in size or, if they do changes certainly are not as obvious as they appear to be in the capon. As concerns weight we find that many of our sinistrally ovariotomized females exceed the weight of the sexless bilaterally ovariotomized birds in this experiment. The average weight of the normal female when one year old is approximately 1400 grams. This weight is not exceeded by the sexless females of this series. The age at the time of operation in these experiments coincides with that at which castration is usually performed in the young male following which there is generally an increase in size. The statements made here are based on general observations and the weights of the birds. No further

commitment can be made until further data are available on this question to be gathered from preparations now being made.

Effect on Accessory Organs.—The term accessory organs is here employed in its customary significance, namely the ducts that convey the products of the gonads to the exterior, the vasa deferentia and the oviducts. In the normal mature male the vasa deferentia are prominent convoluted ducts having a perceptible diameter. Following castration the convolutions are lost accompanied by a marked reduction in size so that one sometimes experiences difficulty in finding these small straight ducts in the adult capon. We have never found any indication of oviducts in the mature Leghorn male. The normal female has but one functional oviduct that on the left side. The right oviduct, present in early embryonic life, degenerates leaving a small rudiment, varying in size in different individuals, attached to the side of the cloaca. The wolffian ducts persist as small slender threads in the normal female. Following ablation of the left ovary the wolffian ducts hypertrophy and frequently become convoluted under the stimulus of the hypertrophying testis-like right gonad (Domm, '27*a*). The oviduct in such cases shows varying degrees of reduction though it is rarely entirely infantile. The highly glandular nature of the oviduct in a certain number of cases of complete sinistral ovariotomy indicates that it is receiving a stimulus in such cases comparable to that furnished by the normal left ovary.

The disposition of the accessory organs in the bilaterally ovariotomized fowl may differ greatly from that found in those merely sinistrally ovariotomized. If the bilateral operation was completely successful in removing all gonad tissue it was found that the wolffian ducts remained small and rudimentary comparable to those found in the normal female (see table, cases no. 875, 876, 882, 887, etc.). The stimulus furnished by very small masses of gonad is apparently insufficient to provoke growth changes as disclosed by case no. 878 (see table). In cases where the mass of regenerated gonad is of considerable size the wolffian ducts have responded to the stimulus furnished by considerable increase in size (see table cases no. 880 and 898). Such a result was to be expected on the basis of the writers earlier observations (Domm, '27*a*). A definite correlation between the amount of hyper-

trophied testis-like gonad present and the growth of these ducts would be difficult to establish though there can be no question as to its existence.

In all cases showing a total absence of gonad the oviduct consists of a small straight flattened tube having a diameter of only 2 to 3 millimeters (see table, cases no. 875, 876, 882, 884, etc.). Even in cases showing small masses of regenerated gonad the oviducts are small and straight and approximate the above in size (see table, cases no. 874, 878, 880, etc.). In only 3 of the cases included in this report were the oviducts other than exceedingly small (see table, cases no. 890, 893, and 894). In each of these cases the oviducts were convoluted and showed a diameter of 4 to 6 millimeters. It should be indicated that in each of the above three cases there is a definite correlation between stimulation of oviduct and reversion to female plumage. In cases no. 878, 884, 887, 899, and 902 (see table), different parts of the oviduct were inflated, to varying degrees, with a clear watery fluid. This condition is not uncommon in our ovariotomized birds and is probably associated with the atrophy of the oviduct in conjunction with the obstruction of both openings thereby preventing the escape of secreted fluids. Our practice of resecting all or a large part of the infundibulum prior to sinistral ovariotomy is no doubt responsible for sealing this end of the oviduct. Rudiments of the right oviduct were found in all cases attached to the side of the cloaca. These rudiments are very small in all of the cases belonging to this series though whether they show a greater reduction in these birds than they do in the normal or the sinistrally ovariotomized fowl would be difficult to estimate. In both our series of sinistrally ovariotomized fowl (Domm, *'27a,* and '28) we encountered many right rudimentary oviducts that were larger than the ones found in the present series but because of the great variation revealed by these structures this is probably not very significant.

Discussion.

The gonadless male and female of the Leghorn variety have a great many points of similarity. In both types the head furnishings remain small and pale and fluctuate little, if any, in size. Both types develop a brilliant, luxuriant, male plumage. The capon

develops long spurs which become sharp and pointed early in their development. The spurs of the gonadless female likewise become long though it appears that they become sharp and pointed somewhat later than those of the capon. The behavior of both types is neutral, neither exhibits the behavior of the normals of either sex. The wolffian ducts, which in the normal male are prominent and convoluted, become very small and straight in the capon so that it is frequently difficult to find them. These ducts are likewise very small and straight in the gonadless female and frequently very difficult to demonstrate. Her oviduct is also greatly reduced to a straight slender tube. The only apparent difference between these two types is that of size. The normal male of the Leghorn variety is larger than the female. Following early castration the male increases somewhat in size as compared with the normal. It is questionable whether the female, bilaterally ovariotomized at a corresponding age, increases in size above the normal. Hence the normal size differences between the sexes appear to persist and may even become somewhat aggravated owing to the increase in size of the capon above the normal male. Studies are now in progress to determine skeletal changes in the castrates of this breed.

The earlier experiments of the writer (Domm, '27*a*) and others have revealed the striking capacity of the female to assume male characters both in anatomy and behavior. These investigations have further shown that the female fowl possesses tissues in the hypertrophied right gonad, which are similar in their effects to the endocrine cells of the testes, upon which this transformation in large measure depends. The experiments of Goodale ('16) Zawadowsky ('22) Finlay ('25) and of ourselves (Domm, '28) reveal the fact that the male may undergo a corresponding transformation of male into female only by operative interference. Hence by grafting ovary into the castrated male such an individual may assume the plumage and head furnishings of the female. The present experiments further reveal the striking identity of the gonadless bird whether originally male or female. The results of castration thus lead to a type common to both sexes designated as the asexual or neutral type by various authors. Lipschutz ('24) maintains that during embryonic life the soma in birds is asexual,

and that the development of male and female characters takes place only under the influence of the sex specific hormones produced by the gonads. Zawadowsky ('22) on the basis of extensive work in the bird concludes that the soma of the male and female is essentially identical, and that differentiation is brought about only by the stimulus of sex specific hormones. He asserts that removal of the gonads leads to an asexual type hence the soma of either sex is "equipotential." Zawadowsky ('26) further reminds us that the development of the right rudimentary gonad in poulardes brings forth a morphogenetic reaction which is an indication of the bisexual nature of the hen. He maintains that not only is the somatic body potentially bisexual but that the left ovary and the right rudimentary gonad of the hen can both produce both male and female morphohormones presumably under given conditions. Furthermore these gonads may produce a typical testicular structure, with active spermatogenesis not infrequently occurring in the activated right gonad (cf. Benoit, '23, Zawadowsky, '26, Domm, '29). Hence Zawadowsky's theory of equipotency would include not only the somatic tissues but also the gonads and presumably the germ cells. Crew ('23) infact postulates equipotency of the primordial germ cells of both sexes. Greenwood's and Crew's ('25) assertion of a difference in intensity, or quantative difference, in male and female hormone and not a sex specific or qualitative difference as is postulated by Lillie ('27), Lipschutz ('24), Zawadowsky ('22 and '26) and others would in addition imply equipotentiality of the hormone secreting cells.

According to Lillie ('27) the real issue is, " what tissues of the male and of the female react equally to the two hormones, whether with respect to growth or alternate potentialities? " Our earlier experiments (Domm, '27*a*) and those of others have shown that in the female fowl the head furnishings, feathers, spurs, wolffian ducts, and to a certain degree the behavior, permanently retain the capacity to react to the male hormone as the corresponding characters of the male normally do. Feminization experiments by ourselves, and others, reveal a similar double potentiality on the part of the corresponding characters of the male. The above observations thus seem to show that the somatic tissues may react equally in both sexes to either male or female hormone while

Crew would include germ cells and Zawadowsky gonads and germ cells also. However as regards equipotency of gonad tissues the theory can apply only to the female and not to the male since no one has ever observed male gonad give rise to ovarian cortex in birds, or mammals for that matter, in spite of the numerous castration and transplantation experiments that have been performed.

If we accept the doctrine of equipotency in its fullest meaning as implied by Zawadowsky, Lipschutz, and others, should we then expect complete sex reversal in cases where the hormone is present in early embryonic life prior to the onset of sexual differentiation? If this is implied these authors would ignore the efficacy of the genetic sexual constitution as factors of differentiation for all extragonadal characters in the presence of the hormones. The observations of Lillie ('17 and '23) on the free-martin reveal a situation in which the production of the sex hormone for the male is demonstrated from the earliest period of sex differentiation. Lillie examined a case of a free-martin in which fusion of the membranes, according to his reconstruction of the probable history of this case, was possibly complete at least at the 10 mm. stage and a vascular anastomosis must have been established at the same time. Such a case, according to Lillie, would seem to have afforded the maximum opportunity of masculinization by the hormones of the male partner on account of the early time of onset and the long duration of possible action. However the modification of the free-martin in this case was not particularly extreme. Lillie ('23) says: "If there were no other factors at work in determining the sex differentiation of embryonic primordia than the specific sex hormone, it is difficult to understand why the free-martin, which receives only male sex hormones, should not become completely male." The chick embryo seemed to offer suitable material for a demonstration of the action of sex hormones on relatively early stages of the developing embryo. Minoura ('21) grafted gonad onto the chorio-allantoic membrane of developing chick embryos. His results seemed to show a definite modification of the female reproductive system in the male direction under the influence of an engrafted testis. Subsequent experiments by Greenwood ('25) Willier ('27) and Willier and Yuh ('28) would seem to show that gonad grafts on the chorio-allantoic membrane

do not exert a specific effect on the reproductive system of the host embryo as maintained by Minoura. The criticism that these grafts had to be made in the second week of incubation when sexual differentiation had already begun is perhaps not very weighty.

Present experiments do not justify the conclusion that sex hormones are absent or are not involved in sexual differentiation in the chick embryo; yet observations on the action of sex hormones in the fowl after hatching make it difficult to accept such a conclusion. Our present evidence on the participation of sex hormones in the development of sexual characters is in evident conflict. The observations of Lillie ('17) on the free-martin, those of Burns ('25) and Witschi ('27) on parabiotic twins in amphibia, and those of Burns ('27) on the effects of gonad grafts in amphian larvæ, furnish evidence for the participation of sex hormones in the embryonic development of sexual characters. On the contrary the observations of Greenwood ('25), Kemp ('25 and '27), Willier ('27), and Willier and Yuh ('28), on gonad grafts in the chick embryo, as well as those of Humphrey ('27) and Witschi ('27) on gonad grafts in amphibian larvæ, furnish negative evidence.

The theory of equipotentiality should receive a more rigorous test than it has hitherto received. There is no question of an apparently equal reaction capacity in males and females of feather germs, head furnishings, spurs, in short all the more obvious external secondary sex characters, to the presence of ovary or testis. The same thing may be true of the sexual ducts though the evidence is less conclusive; there is also evidence that sex behavior is strongly influenced by the heterologous sex hormones in the parallel direction. However the most interesting and fundamental question suggested by this work is whether the earliest lines of germ cells are also equipotential and capable of forming ova or spermatozoa according to internal environmental conditions. Benoit's ('23) implication is that they are not equipotential. He explains his cases of sex transformation in the female by assuming the presence of two distinct germ lines in the female, the male line, in the medulla of the ovary, and the female line, in the cortex "the one as rigorously fixed as the other from the point of view

of their cyto-sexual determinism." Our recent experiments (Domm, '29) have confirmed the occurrence of spermatogenesis following ovariotomy in the fowl and explained the causes of its occurrence. It seems to us more reasonable to believe that the primordial germ cells, of the female at least, are equipotential, and that their ultimate fate as male or female is determined by environmental exigencies; hence, when they become incorporated in the cords of the medulla they produce spermatogenesis and when in the cortical elements of the gonad they produce ovogenesis. This agrees with Witschi's ('29) interpretation of sex reversal in female tadpoles following the application of high temperature.

Are the endocrine cells of the gonad also equipotential and thus capable of producing male or female secretions according to environmental exigencies? Our present indications are that these cells are of two kinds, the male secreting and the female secreting. The female possesses both, the male secreting cells in a reserve of specific tissue the medulla, normally inhibited by the cortex, but capable of growth and secretion when this inhibition is removed (Domm, '27*a*, '28, '29), and the female secreting cells in the cortical elements of the gonad. The male possesses but one the male secreting cells. Our experimental results (Domm, '27*a* and unpublished data) demonstrate quite clearly that male hormone may be produced either by testis or ovarian medulla but that female hormone is produced only by ovarian cortex. There is therefore no indication of equipotentiality of these cells and according to Lillie ('27) "none is to be expected, seeing that these cells are the source of the postulated inductions of the double potentialities."

Summary.

1. Complete bilateral ovariotomy in the brown leghorn fowl leads to an asexual or neutral type common to both sexes in many of its characters.

2. The head furnishings which become large and male-like following sinistral ovariotomy remained small and fluctuated little in size following complete bilateral ovariotomy.

3. Following sinistral ovariotomy the plumage becomes male but at a later period, varying greatly in different individuals, it reverts to the female type. In our cases of complete bilateral ovariotomy

the plumage became male following the operation and retained this character to the termination of the experiment.

4. Well developed spurs were found in all cases. The amount of spur tissue developed does not seem to be greater in the bilaterally ovariotomized fowl than in those sinistrally ovariotomized.

5. The behavior of these individuals is neither male nor female but neutral. Comparable in this respect to that of the capon.

6. The Wolffian ducts hypertrophy following sinistral ovariotomy. No such hypertrophy is perceptible in the bilaterally ovariotomized fowl, these ducts being small, straight and often very difficult to find.

7. The amount of oviduct tissue varies greatly in the sinistrally ovariotomized fowl. In the cases of complete bilateral ovariotomy here recorded the oviduct is reduced to a very small straight flattened tube, 2–3 mm. in diameter. Very small rudiments of the right oviduct were found in all cases.

8. No changes were observed in size. The birds retained approximately the size of normal hens.

Explanation of Table on Bilateral Ovariotomies.

The table includes cases of complete as well as incomplete bilateral ovariotomy. The complete cases are those in which no gonad regenerated on either right or left sides as determined by post-mortem examination. In incomplete cases masses of gonad varying in size are found on either right or left sides or both. On account of the length of the records each case is continued on a second page.

The record of each case consists of selections, from very much more complete records, considered to be most important for the operation history. In some cases other data are recorded in the text. The preserved records consist of notebooks containing complete histories of all birds, photographs, feather records for each case, skins, preserved Sacrums with the urinogenital organs in situ for each case, and other anatomical preparations. All enteries have been checked thrice from the original records.

Column 1 gives the identification number of each bird.

Column 2 gives the age of the bird in days at the time of the first sinistral operation. It also gives the dates of the sinistral and dextral operations in their order. The sinistral operation always preceded.

Column 3 gives the date of death and autopsy and, if the bird was found dead, this fact and the cause of the death, if known. Cb. signifies crop-binding revealed at post-mortem.

†. A "secondary operation" for removal of regenerated gonad.

"Successive changes in plumage" and "successive changes in head furnishings" are recorded at 3 months, 6, 12, 18, 24, and 30 months following

the date of the operation. These periods are not always the best for recording changes, hence observations at other times are frequently entered in the nearest column and indicated by a number in parenthesis giving the actual age in months above the individual entry. All such dates are approximate only, but on account of the relative slowness of the changes they are sufficiently exact.

Plumage Changes.—The changes recorded are, in general, the natural plumage changes, not forced by plucking. The only exceptions are operation sites though, because of the rapid continuous development of male plumage in most of these cases, these are not long apparent.

♂ indicates feathers of cock or capon type not distinguished.

♀ indicates feathers of female type.

"Tipped" always refers to feathers with female tip and male base which appear shortly after ovariotomy; these feathers have commonly a very sharp line of demarcation between the components, and are frequently referred to as "gynandromorph" feathers in the literature. Such feathers may begin to appear in 10 to 14 days after a successful operation, and they are commonly abundant at 3 months interspersed with new completely male feathers. I. signifies "intermediate," and represents the beginning of the secondary transformation from the male to the female type of feathers in these birds. These feathers may have male tips and female bases, but the transition zone is not sharp but diffuse. In some instances the entire feather is intermediate or of this diffuse nature.

Where regions are indicated, abbreviations are used Br. for breast, Ba. for back, Sa. for saddle, T. for tail, W's. for wings, Wc's. for wing coverts, Juv. for juvenile, etc.

Head Furnishings (measurements are in centimeters).—The comb is given first, the length of the main blade of the comb from front to back being the numerator and the greatest depth from the highest point to the base the denominator; the wattles come second, width over depth; the vertical diameter of the ear-lobe comes last.

Spurs.—Spurs are recorded by their length in centimeters at date of autopsy.

Findings at Autopsy.—At the time of autopsy, the head was preserved separately in formalin, the skin with, or without, legs attached removed, cured and preserved, and the entire sacrum with urinogenital organs including gonads, if present, fixed in Bouin's fluid.

Right and Left Gonads.—None signifies no gonad, T. signifies "testis-like" gonad macroscopically, † see column 3. Measurements are length over transverse diameter, in centimeters. These 'regenerated' gonads are less irregular than the normals frequently are hence the measurements are fairly good comparative estimates of volume in these cases.

Right and Left V. D. (vas deferens).—For purposes of succinct characterization the arbitrary scale previously devised (Domm, *'27a*) was utilized in which 1 corresponds to the condition of the normal right vas deferens in the female and 5 that of the male; 2, 3, and 4 represent intermediate conditions; 2, wide, straight; 3, slightly convoluted; 4, strongly convoluted. Observations are more difficult to make on the left side on account of accumulations of fat in the mesentery of the oviduct where the

vas lies; a question mark in this column indicates only that the observation could not be made owing to fat (*e.g.*, 875, 882, 884, etc.).

Oviduct.—Similarly, a scale of 6 points was adopted for recording variations of the left oviduct. 1, the most reduced type, straight and only 2–3 mm. in diameter; 2, straight, 4 mm. or more in diameter; 3, convoluted, 3–5 mm. in diameter; 4, convoluted, 6–9 mm. in diameter; 5, convoluted, 10 + mm. in greatest diameter; 6, oviduct of a normal laying hen (see Domm, '27*a*, compare plate 8, Fig. 2*b*; plate 9 and 10; also plate 11, no. 729). * Varying portions of oviduct inflated with fluid (see text page 19).

SUMMARY OF BILATERAL OVARIOTOMIES.

Bird No.	Operation age and Date.	Autopsy Date.	Successive Changes in Plumage.						Spurs.	
			3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt.	Lt.
874	76 d. 9–14–26 9–30–26	Died 4–16–28	Predom. ♂, some Juv. Some old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. New ♂.	♂ in all areas New ♂.	♂ in all areas. New ♂.	(19) ♂ in all areas. New ♂.		2.0	1.9
875	76 d. 9–14–26 9–30–26	Died 10–11–28	Predom. ♂. Many Juv. Some old ♀ and ♀ Tp'd. New ♂	♂ in all areas, few scat. old ♀ WC's. New ♂.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(25) ♂ in all areas. New ♂.		1.8	1.9
876	76 d. 9–14–26 9–30–26	Died 11–11–28	Predom. ♂. Many Juv. prin. on Ba. and Sa. Some old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. Some Juv. on Ba. and Sa. few scat. old ♀ WC's. New ♂.	♂ in all areas. New ♂ numerous. Molting.	♂ in all areas. New ♂.	(26) ♂ in all areas. New ♂.		2.3	2.3
878	76 d. 9–14–26 9–30–26	Died 7–20–27	Predom. ♂, few Juv. on Ba. Sa. and W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. New ♂.	(10) ♂ in all areas. New ♂.				1.7	1.7
880	76 d. 9–14–26 9–30–26	11–2–27	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. Some old ♀. few I. New ♀.	(8) Predom. ♂. ♀ bec. numerous. few I. New ♀.	(12) Predom. ♂. many ♀. few I. New ♂.	(13 1/2) Predom. ♂. few Scat. ♀ and I. New ♂.		0.5	1.1

Bird No.	Successive Changes in Head Furnishings.							Findings at Autopsy.				
	At Operation.	3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt. Gonad.	Lt. Gonad	Rt. V.D.	Lt. V.D.	Lt. Ovd.
874	2.1 1.0 / 1.1 / 0.6 0.4	2.6 1.6 / 1.4 / 1.4 0.6	3.6 2.0 / 1.6 / 2.1 1.1	5.2 2.4 / 1.6 / 2.5 1.9	5.2 2.2 / 1.7 / 1.9 1.7	(19) 4.8 1.9 / 1.6 / 1.7 1.3		None	T. 1.5 / 0.5	1–2	1–2	1
875	1.0 1.3 / 1.1 / 0.5 0.3	2.5 1.6 / 1.4 / 1.1 0.6	2.8 1.5 / 1.5 / 1.4 0.5	3.0 1.9 / 1.5 / 1.4 0.9	3.0 2.0 / 1.5 / 1.3 1.0	(25) 2.8 2.0 / 1.4 / 1.1 1.0		None	None	1	?	1
876	2.1 1.5 / 1.1 / 0.6 0.4	2.9 1.9 / 1.2 / 1.4 0.6	2.7 1.5 / 1.3 / 1.4 0.5	4.3 1.7 / 1.5 / 1.6 1.3	4.3 1.9 / 1.5 / 1.6 1.3	(26) 4.1 2.1 / 1.4 / 1.4 1.1			None	1	1	1
878	2.1 1.3 / 1.0 / 0.7 0.4	2.6 1.5 / 1.5 / 1.4 0.6	3.7 2.0 / 1.6 / 2.3 1.2	(8) 4.5 2.1 / 1.8 / 2.8 1.5	(10) 4.2 1.9 / 1.6 / 2.4 1.0			None	T. 1.1 / 0.3	1	1	1 *
880	2.1 1.3 / 1.1 / 0.9 0.4	2.8 1.5 / 1.6 / 1.4 0.6	6.9 3.3 / 2.4 / 3.9 3.0	(8) 8.9 3.9 / 2.8 / 5.1 4.1	(12) 6.8 2.6 / 2.5 / 3.9 3.0	(13½) 5.7 2.1 / 2.3 / 2.6 2.3		None	T. 1.2 / 0.3	2	2	1

Bird No.	Operation age and Date.	Autopsy Date.	Successive Changes in Plumage.						Spurs.	
			3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt.	Lt.
882	78 d. 9–16–26 9–30–26	3–2–29	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. Some old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. Some Juv. on Ba. and Sa. New ♂.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(25) ♂ in all areas. New ♂.	(29½) ♂ in all areas. New ♂.	2.0	1.4
884	78 d. 9–16–26 10–6–26	2–8–29	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. few Juv. few old ♀ on W'S. New ♂.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(25) ♂ in all areas. New ♂.	(28¾) ♂ in all areas. New ♂.	3.0	3.3
887	78 d. 9–16–26 10–5–26	3–1–29	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. few old ♀ on W'S. New ♀.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(25) ♂ in all areas. New ♂.	(29½) ♂ in all areas. New ♂.	1.0	1.5
888	78 d. 9–16–26 10–5–26	Died 12–6–26	(2¾) Predom. ♂. Many Juv. on Ba. Sa. and W'S. Some old ♀. New ♂.						0.2	0.2

Bird No.	Successive Changes in Head Furnishings.							Findings at Autopsy.				
	At Operation.	3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt. Gonad.	Lt. Gonad.	Rt. V.D.	Lt. V.D.	Lt. Ovd.
882	2.2 1.0 — — 1.2 0.9 0.7	2.9 1.6 — — 1.2 1.4 0.7	2.9 1.7 — — 1.6 1.5 0.7	3.1 1.8 — — 1.8 1.4 0.8	2.9 1.9 — — 1.7 1.3 0.8	(25) 3.0 1.9 — — 2.0 1.3 1.0	(29½) 2.9 1.8 — — 1.7 1.2 1.0	None	None	1	?	1
884	2.0 1.4 — — 0.8 0.7 0.3	2.5 1.5 — — 1.4 1.0 0.5	2.7 1.4 — — 1.2 1.1 0.4	2.7 1.4 — — 1.3 1.1 0.4	2.9 1.4 — — 1.6 1.1 0.7	(25) 2.8 1.5 — — 1.4 1.0 0.6	(28¾) 2.8 1.5 — — 1.4 0.9 0.5	None	None	1	?	1 *
887	1.8 1.2 — — 0.9 0.7 0.3	2.5 1.6 — — 1.2 1.4 0.6	4.6 2.0 — — 1.5 2.5 1.3	4.0 1.7 — — 1.5 2.1 0.9	4.1 1.8 — — 1.4 1.6 1.0	(25) 4.1 1.9 — — 1.5 1.7 1.1	(29½) 4.1 1.7 — — 1.6 1.6 1.1	None	None	1	?	1 *
888	2.0 1.4 — — 0.8 0.8 0.3	(2¾) 2.9 1.8 — — 1.1 1.1 0.4						None	None	1	?	1

Bird No.	Operation Age and Date.	Autopsy Date.	Successive Changes in Plumage.						Spurs.	
			3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt.	Lt.
890	78 d. 9–16–26 10–5–26	8–12–27	Predom. ♂. few Juv. on Ba. and W'S. Many old ♀. Some ♀ Tp'd. New ♂.	Predom. ♀. Many scat. ♂. Some I. New ♀.	(8) Predom. ♀. Many Scat. ♂. Some I. New ♀.	(11) aprox. ½♂ ½ ♀. Some I. New Ba. ♂.			0.3	0.3
891	78 d. 9–16–26 10–5–26	3–1–29 †	Pract. all ♂. Many Juv. few scat. old ♀ and ♀ Tp'd. New ♂.	Predom. ♂. Many new ♀. Some I. New ♀.	Aprox. ½♂ ½ ♀. New ♂.	♂ in all areas. New ♂.	(25) ♂ in all areas. New ♂.	(29½) ♂ in all areas. New ♂.	2.7	2.7
893	79 d. 9–17–26 10–5–26	7–23–27	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. few old ♀ and ♀ Tp'd. New ♂.	Predom. ♂. Some I and new ♀. New ♀.	(10¼) Predom. ♂. Many ♀. Some I. New ♀.				1.3	1.3
894	79 d. 9–17–26 10–6–26	5–6–29	Pract. all ♂. Some Juv. on Ba. Sa. and W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. Some Juv. on Ba. and Sa. New ♂.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(25) Predom. ♂. Many ♀. few I. New ♀.	(31½) Predom. ♀ in all areas. few scat. ♂ and I. New ♀.	4.4	Lost

Bird No.	Successive Changes in Head Furnishings.							Findings at Autopsy.				
	At Operation.	3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt. Gonad.	Lt. Gonad.	Rt. V.D.	Lt. V.D.	Lt. Ovd.
890	2.1 1.3 / — / — 1.0 / 0.8 0.3	2.6 1.4 / — / — 1.3 / 1.2 0.3	7.4 3.2 / — / — 2.2 / 3.6 3.1	(8) 9.4 4.0 / — / — 2.6 / 4.3 4.2	(11) 6.8 2.6 / — / — 2.1 / 2.8 2.8			None (See text p. 8)	None	1	1	3
891	2.3 1.4 / — / — 1.0 / 1.0 0.3	3.1 1.7 / — / — 1.2 / 1.7 0.5	(7) 9.5 3.7 / — / — 2.4 / 5.4 3.6	5.1 2.4 / — / — 2.2 / 2.6 2.2	4.8 2.2 / — / — 2.0 / 2.3 1.6	(25) 4.6 2.3 / — / — 2.0 / 2.2 1.8	(29½) 4.5 2.2 / — / — 1.8 / 2.2 1.8	† 10–16–27 T. Small Cauterized. . . . . 3–1–29 None	None	1	?	1
893	2.2 1.3 / — / — 1.0 / 1.1 0.3	2.7 1.6 / — / — 1.4 / 1.6 0.6	7.7 3.5 / — / — 2.1 / 5.1 3.4	(8) 9.3 4.3 / — / — 2.6 / 6.1 4.8	(10¼) 5.4 2.3 / — / — 1.8 / 2.9 2.2			T. 0.6 / — / 0.3	T. 0.8 / — / 0.2	1	1	3
894	1.8 1.4 / — / — 0.8 / 0.5 0.3	2.6 1.8 / — / — 1.3 / 1.2 0.4	3.0 1.6 / — / — 1.3 / 1.4 0.4	3.6 1.7 / — / — 1.6 / 1.4 1.0	5.7 2.3 / — / — 1.9 / 2.5 1.8	(25) 9.0 3.3 / — / — 2.2 / 4.3 3.2	(31½) 10.8 3.8 / — / — 2.3 / 5.9 3.8	T. 1.5 / — / 1.1	T. 0.9 / — / 0.9	3	3	4

Bird No.	Operation age and Date.	Autopsy Date.	Successive Changes in Plumage.						Spurs.	
			3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt.	Lt.
895	79 d. 9–17–26 10–6–26	3–1–29	Pract. all ♂. Many Juv. on Ba. and Sa. few on W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. Some Juv. on Ba. and Sa. New ♂.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(25) ♂ in all areas. New ♂.	(29½) ♂ in all areas. New ♂.	3.0	3.0
897	79 d. 9–17–26 10–5–26	9–29–27	Pract. all ♂. Many Juv. on Ba. and Sa. few on W'S. Some old ♀ WC'S. New ♂.	♂ in all areas. exc. few old ♀ WC'S. New ♂.	(12½) ♂ in all areas. New ♂.				1.4	1.2
898	79 d. 9–17–26 10–5–26	Died 6–7–27 Cb.	Pract. all ♂. Many Juv. on Ba. and Sa. few on W'S. few old ♀ and ♀ Tp'd. New ♂.	♂ in all areas. exc. few old ♀ Tp'd. WC'S. New ♂.	(8½) ♂ in all areas. New ♂.				1.6	1.6
899	79 d. 9–17–26 10–6–26	2–24–27	Pract. all ♂. Many Juv. on Ba. Sa. and W'S, few old ♀ and ♀ Tp'd. New ♂.	(5¼) ♂ in all areas. Many Juv. few old ♀ WC'S. New ♂.					0.6	0.6

Bird No.	Successive Changes in Head Furnishings.							Findings at Autopsy.				
	At Operation.	3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt. Gonad.	Lt. Gonad.	Rt. V.D.	Lt. V.D.	Lt. Ovd.
895	2.0 1.4 / — — 1.0 / 0.7 0.3	2.6 1.6 / — — 1.4 / 1.4 0.6	3.1 2.0 / — — 1.5 / 1.6 0.5	3.3 2.0 / — — 1.6 / 1.5 0.8	3.4 2.0 / — — 1.7 / 1.3 1.0	(25) 3.5 2.0 / — — 2.1 / 1.3 1.2	(29½) 3.5 2.0 / — — 2.0 / 1.3 1.1	None	None	1	1	1
897	2.1 1.2 / — — 0.8 / 0.9 0.4	2.8 1.4 / — — 1.2 / 1.2 0.6	3.2 1.6 / — — 1.2 / 1.5 0.7	(9) 3.2 1.6 / — — 1.3 / 1.6 1.0	(12½) 3.2 1.6 / — — 1.3 / 1.7 0.8			None	None	1	1	1
898	1.9 1.2 / — — 0.9 / 0.7 0.3	2.6 1.6 / — — 1.3 / 1.3 0.7	8.1 2.4 / — — 1.7 / 3.2 1.8	(8½) 5.2 2.1 / — — 1.4 / 2.4 1.4				T. 0.3 / — / 0.2	T. 1.0 / — / 0.2 +	3	3	1
899	2.0 1.1 / — — 0.9 / 0.9 0.2	2.7 1.4 / — — 1.3 / 1.1 0.3	(5¼) 2.5 1.2 / — — 1.2 / 1.2 0.2					None	None	1	1	1 *

Bird No.	Operation age and Date.	Autopsy Date.	Successive Changes in Plumage.						Spurs.	
			3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt.	Lt.
902	79 d. 9–17–26 10–5–26	Died 7–4–27	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. Some old ♀ and ♀ Tp'd. New ♂.	Predom. ♂ .few Juv. few old ♀ WC'S. New ♂.	(9½) ♂ in all areas. New ♂.				0.5	0.5
903	79 d. 9–17–26 10–6–26	6–3–27	Predom. ♂. Many Juv. on Ba. and Sa. few on W'S. Some old ♀ and ♀ Tp'd. New ♂.	Predom. ♂. Some Juv. on Ba. Sa. and W'S. few old ♀ WC'S. New ♂.	(8½) ♂ in all areas. Some Juv. on Sa. and W'S. New ♂.				1.1	1.1

Bird No.	Successive Changes in Head Furnishings.							Findings at Autopsy.				
	At Operation.	3 months.	6 months.	12 months.	18 months.	24 months.	30 months.	Rt. Gonad.	Lt. Gonad.	Rt. V.D.	Lt. V.D.	Lt. Ovd.
902	1.9 1.2 / — — / 0.7 0.3 1.1	2.5 1.7 / — — / 1.2 0.3 1.4	2.8 1.4 / — — / 1.7 0.3 1.6	(9½) 2.9 1.6 / — — / 1.7 0.3 1.5				None	None	1	1	1 *
903	2.0 1.1 / — — / 0.8 0.2 1.1	2.4 1.3 / — — / 1.1 0.3 1.3	2.7 1.4 / — — / 1.2 0.3 1.3	(8½) 2.8 1.4 / — — / 1.3 0.3 1.3				None	None	1	1	1

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PLATE I.

Explanation of Figures.

No. 1018. Poulard *Sinistrally ovariotomized.* This bird was hatched on May 9, 1927, and sinistrally ovariotomized on May 22, 1927, when 13 days old. This photograph was taken on May 22, 1929, 2 years following the operation. The bird at this time was completely male plumaged, showed well developed masculine head furnishings and long spurs. The new ingrowing feathers at this time were intermediate heralding the inevitable change to female plumage which apparently all these birds ultimately undergo.

No. 845. Poulard *Sinistrally ovariotomized.* This bird was hatched on June 16, 1926 and sinistrally ovariotomized on August 11, 1926, when 56 days old. This photograph was taken on May 22, 1929, approximately two years and nine months following the operation. The bird at this time was completely female plumaged though it showed prominent masculine head furnishings, spurs, and behavior. The definitive condition thus is one in which the sinistrally ovariotomized fowl becomes female plumaged while she retains her other acquired male characters. Complete dextral ovariotomy in such a bird brings about the reassumption of male plumage and a loss of the dependent sexual characters leading to the asexual capon type (see Domm, '27*a*). (Compare bird no. 876, Plate 2.)

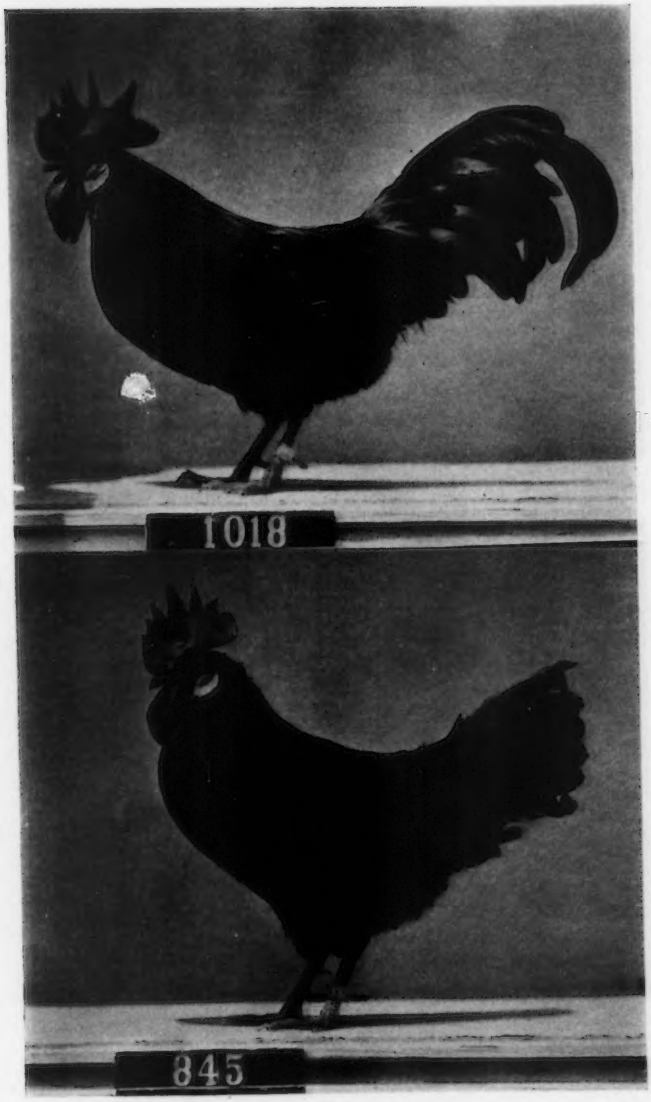

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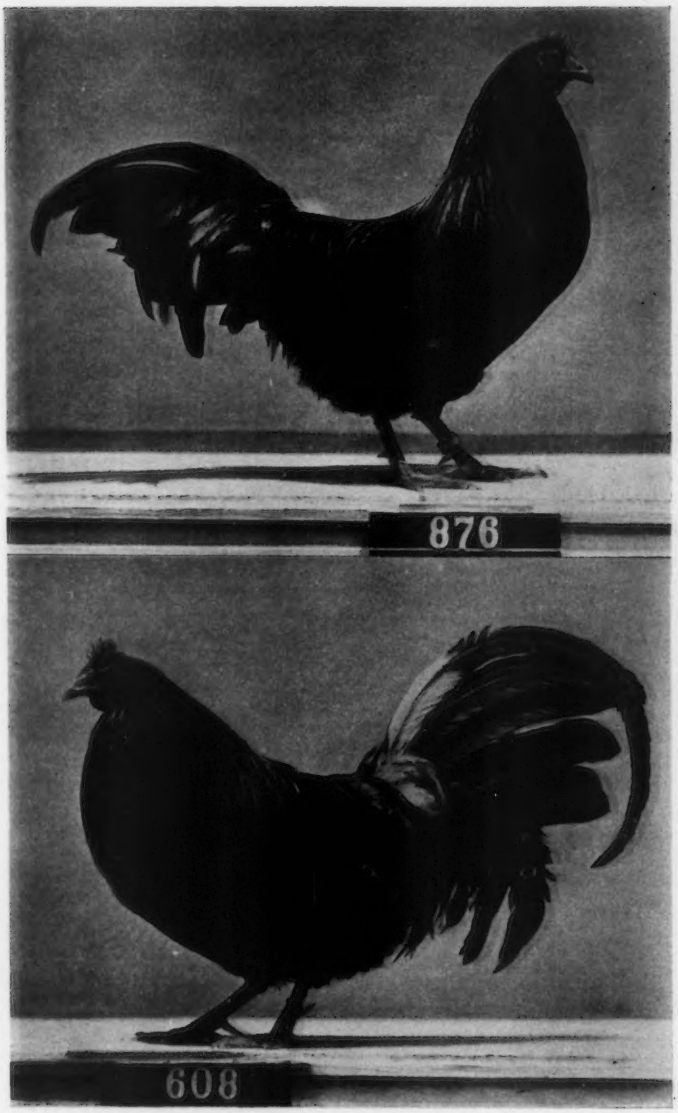
PLATE II.

Explanation of Figures.

No. 876. Poulard *bilaterally ovariotomized*. For detailed history see table. This bird was hatched on June 30, 1926. A sinistral ovariotomy was performed on September 14, 1926, when the bird was 76 days old and 16 days later on September 30, a dextral operation was performed destroying the right rudimentary gonad by electric cauterization. This photograph was taken on June 11, 1928, 1 year and 9 months following the operation. Its appearance was typically capon showing luxuriant male plumage, small head furnishings, well developed spurs, and neutral behavior (compare bird no. 608 this plate). The bird retained these characters up to the time of its death on November 11, 1928. Post-mortem examination revealed no gonad tissues on either right or left gonad sites.

No. 608. Capon. This bird was hatched on April 15, 1927. Its left testis was removed on May 7, 1927, when 22 days old; the right testis on June 9, 1927, 33 days later. This photograph was taken on May 22, 1929. The bird was killed on May 24, 1929, post-mortem examination revealed no gonad tissue. The bird had been a typical capon during the entire period it was under observation showing luxuriant male plumage, small head furnishings, well developed spurs, and neutral behavior.



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